HYPOTHALAMUS, PITUITARY AND THYROID

the control system of thyroid hormone production

w. j. sluiter

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STELLINGEN

I

De hoogte van de basale serum TSH-spiegel bij primaire hypothyreoidie weerspiegelt niet de mate van hypothyreoidie.

II

De verhouding tussen de basale serumspiegel van het TSH en de response daarvan op TRH-stimulatie loopt parallel met de ernst van de hypothyreoidie bij primair schildklierlijden

III

De overproductie van schildklierhormoon bedraagt reeds 25% of meer, als een response van het serum TSH op TRH-stimulatie uitblijft.

IV

Bij saturatieanalyse van hormoonreceptoren dient men zich te realiseren, dat ook andere onderdelen van het gebruikte preparaat affiniteit voor het hormoon zouden kunnen bezitten. Vergaande interpretatie van zgn. "Scatchard-plots" betreffende affiniteit en aantal is daarom misleidend.

V

Gezien de kinetiek van het "glucocorticoied-effect" op de perifere T_3-productie uit T_4, wordt de activiteit van het betreffende enzyme waarschijnlijk gereguleerd door phosphorylering-dephosphorylering.

VI

Wanneer folliculair of papillair schildkliercarcinoomweefsel het accumulerend vermogen voor jodide heeft verloren, betekent dit geenszins dat andere functies voor dit weefsel niet meer onder TSH-invloed staan. Dit betekent dat hormonale suppressie-therapie rationeel blijft.

VII

Bij beoordeling van endocriene functietesten mag „voldoende" niet verwisseld worden met „normaal".

VIII

Bij de chirurgische behandeling van de ziekte van Cushing zal adrenalectomie worden verdrongen door transsphenoidale microchirurgie van de hypophyse.

IX

Het is noodzakelijk dat internisten in opleiding gelegenheid wordt geboden zich via een stageperiode binnen de opleidingstijd te verdiepen in de aspecten van laboratoriumbepalingen betreffende sensitiviteit, specificiteit, storingsgevoeligheid en kosten.

X

De voorspellende waarde van een „diagnostische test" met betrekking tot een bepaalde diagnose, hangt sterk af van de "prevalence of the disease" in de voor deze test aangeboden patientengroep en wordt daarom zeer sterk bepaald door de kundigheid van de aanvrager.

XI

Gezien de lange duur van de te leveren krachtsinspanning kent de internationale zwemsport geen sprintnummers. Internationale erkenning van 50 meter-nummers is daarom gewenst.

XII

Beschermende maatregelen ten behoeve van met uitsterven bedreigde diersoorten hebben slechts kans op succes, wanneer ze geënt zijn op het toekennen van een beheerdersfunctie aan de aanvankelijke bedreigers.

Stellingen

behorende bij het proefschrift van

W. J. SLUITER

Hypothalamus, pituitary and thyroid

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RIJKSUNIVERSITEIT TE GRONINGEN

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The control system of thyroid hormone production.

PROEFSCHRIFT

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WILLEM JACOBUS SLUITER

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DRUKKERIJ VAN DENDEREN B.V.
GRONINGEN

Promotor:Prof. Dr. H. Doorenbos
Copromotor: Prof. Dr. M. G. Woldring

The investigations were carried out under supervision of Prof. Dr. H. Doorenbos in the Division of Endocrinology of the Department of Internal Medicine, University Hospital Groningen, with the help from the members of the medical and laboratory staff of the Division and of the Central Isotope Laboratory (Head Prof. Dr. M. G. Woldring).

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CONTENTS

INTRODUCTION

The idea to start this investigation was provoked by the apparent disagreement between the very wide so called "normal ranges" of the serum levels of the thyroid hormones T_4 and T_3, and the extreme sensitivity of the hypothalamus-pituitary control system to minimal changes in thyroid hormone production. Evidence has been obtained by others, that indeed the variations of the serum levels of T_4 and T_3 in a normal individual are much smaller than is suggested by the total "normal ranges" of the whole population. This means that thyroid dysfunction can be present despite of the finding of serum thyroid hormone levels within the "normal range". Though tests are available nowadays to detect this kind of dysfunction, the knowledge of the control system is too shallow for a correct appreciation of the test results regarding the extent and the gravity of the abnormality. Especially in the patient referred because of cardiac symptoms, a proper judgment of the thyroid function is of extreme importance regarding diagnosis and therapy, because its effect on the myocardium is so pronounced. Even a small under- or overproduction of thyroid hormone may eventually cause or aggravate cardiac dysfunction and should therefore be excluded or otherwise quantitated in these patients.

Chapter 1

THE REGULATION OF THYROID HORMONE SECRETION

1.1 INTRODUCTION

In man as in other warmblooded creatures the level of metabolic activity of most tissues is controlled by thyroid hormone. Thyroid hormone markedly stimulates biochemical pathways that yield energy-rich intermediates, providing an enhanced supply for many energy dependent processes, of which a majority is stimulated as well. This overall turnover rate of energy, clinically expressed as the basal metabolic rate (BMR), has to be controlled to fluctuate within a very narrow range to fulfil the demand of homeostasis. As its influence on basal metabolism is so pronounced, it is obvious that the production of thyroid hormone should be regulated precisely.

The main feature of the regulation system for thyroid hormone secretion is the feed back mechanism; thyroid hormone inhibits the secretion of the stimulator of its own production. The backbone of the regulating system is the hypothalamus-pituitary-thyroid-axis (HPT-axis).

The production of thyroid hormone is accelerated by thyroid-stimulating-hormone (TSH). The release of TSH from the pituitary is stimulated by thyrotropin-releasing-hormone (TRH), but inhibited by thyroid hormone. TRH is produced by the hypothalamus.

Consequently, the pituitary can be considered as a thermostat, in which TRH creates the set point for the thyroid hormone level (see figure 1-1).

Each part of this control system will be described in the next paragraphs.

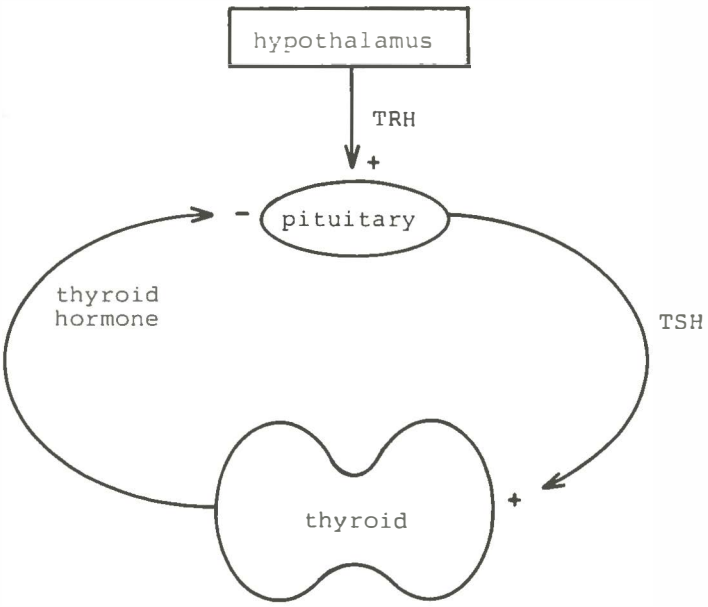


Figure 1.1. The hypothalamus-pituitary-thyroid axis.

1.2 THE HYPOTHALAMUS

The hypothalamus is the part of the brain nearest to the pituitary gland. It is located at the base of the brain, forming the floor of the third ventricle and is bordered by the optic chiasma anteriorly and by the mammillary bodies posteriorly. One specialized region in the floor of the hypothalamus, the median eminence, is connected to the pituitary by means of a stalk.

The hypothalamus controls the secretion of pituitary hormones by way of a complex neural process, ending in the formation and release of several so-called releasing hormones and release-inhibiting hormones, capable of stimulating or inhibiting the release by the pituitary of the appropriate hormones (1,2). The releasing hormone involved in TSH secretion is TRH.

1.2.1 *TRH-production*

TRH is a small molecule, pyroglutamyl-histidyl-prolinamide (see figure 1-2). Its official name is thyroliberin. TRH is formed by hypothalamic tissue, by a non-ribosomal enzymatic system called TRH-synthetase (3).

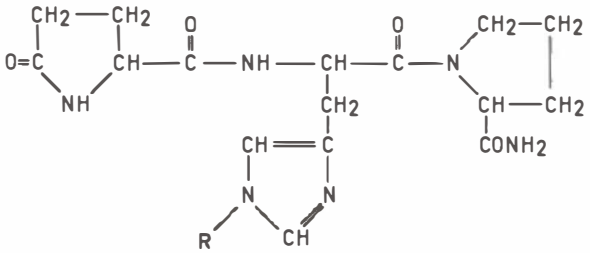


Figure 1-2. Thyrotropin releasing hormones.
R = H; structure of TRH
R = CH_3; structure of N(3im)-methyl TRH

The enzyme system is present in the particulate-free supernatant of the tissue homogenate and requires, besides the three aminoacids glutamic acid or glutamine, histidine and proline, as essential substances Mg^{2+} and ATP.

Of the three aminoacids, histidine and proline probably are connected first, then the glutamyl bond is formed. Intramolecular cyclisation of the glutamyl side chain occurs afterwards (pyro-glutamic acid is not incorporated)(3). At what stage proline is amidated is unknown.

1.2.2 *Distribution of TRH*

Though only formed in the hypothalamus, TRH is present in other brain sections. Even about 70% of the TRH in rats is found outside the hypothalamus, though at concentrations of at least ten times lower than in the hypothalamus. Hardly any TRH is detectable in the cerebellum (4,5).

Because of its distribution over merely all brain sections, its presence in cerebral spinal fluid is understandable (6,7).

TRH is present in the human fetal brain as early as the fourth gestational week (8). In adult man TRH is found in high concentrations in a hypothalamic strip from the posterior nucleus to the pituitary stalk (9). This localisation is remarkably different from the sites of highest concentration of another releasing hormone, luteinising hormone releasing hormone (LHRH). LHRH is found mainly in a strip of hypothalamic tissue leading from the region between supraoptic and preoptic nuclei to the stalk.

1.2.3 *Transfer and release of TRH*

The synthesis of TRH is thought to occur mainly in the cell bodies in the posterior nucleus, from where it is transported down the axons to be stored in the terminal dendrites in the first two thirds of the stalk. This explains the very high concentration of TRH in this part of the stalk (9).

The release of the stored hormone into the hypophyseal portal blood system is probably under neurological control from higher centers, which are involved in daily rhythm and stress.

In vitro, synaptosomes from the median eminence release more TRH in the presence of dopamine, whereas 5-hydroxy-tryptamine blocks this release (10). To be active, dopamine must be converted to norepinephrine (11), which probably stimulates cAMP formation, as dibutyryl cyclic AMP mimics the dopamine action (11).

After release, TRH is transferred to the distal part of the adenohypophysis by the bloodstream of the long portal vessels, originating in the capillary loops of the superior hypophyseal artery. In contrast to TRH, LHRH uses the short portal vessel system as well, originating in the inferior hypophyseal artery (9).

1.2.4 *Fate of TRH*

TRH injected into the blood stream has a very short serum half life of about 5 minutes (12). Only 5% of a dose is excreted in the urine

unaltered; TRH is very rapidly degradated by plasma enzymes. Most likely the first step in the degradation is the cleavage of the histidyl proline bond (13); deamination plays only a minor role (13,14). Whether the enzyme(s) responsible for this action is (are) identical to the converting enzyme involved in the hydrolysis of the histidyl-phenylalanyl bond in angiotensine I is of minor importance.

Strong evidence has been obtained in the rat that the degradating activity in the hypophyseal portal system is only 25% of that of peripheral blood (13). Whether this phenomenon must be explained by assumption of inhibitors present in hypophyseal blood or by inactivation of the enzyme(s) during passage of the capillary loops of the hypophyseal arteries, remains unsolved.

1.2.5 *Control of TRH release*

The balance of dopamine and serotonine probably mainly controls the release of TRH in normals. All processes capable of shifting the dopamine-serotonine balance exert in this way their influence on TRH release. One of these processes surely is the daily sleep-wake cycle. Most of the pituitary hormones are released predominantly during sleep, probably because of enhanced release of releasing hormones. A sleep-wake rhythm of TRH release is present, as will be discussed later on.

Apart from this "normal" daily control, including the sleep-wake cycle, additional influences on TRH release exist. In some animals and in the newborn human, exposure to cold leads to TSH release (15,16), through TRH release (11). In the adult human this response is almost completely lost though still detectable (17). After longer periods of cold exposure, another mechanism of adaption, through enhanced turnover of thyroid hormones, leads to higher TSH levels (18).

In the past, the influence of thyroid hormones on TRH production has been controversial. There is a widespread belief that thyroid hormones enhance TRH production (11). This can be observed when

the thyroid hormone feedback becomes negligible. During the progression of hypothyroidism of long duration after thyroidectomy in rats, the raised serum TSH levels progressively decrease (19). At the same time the pituitary content of TSH, initially lower than normal, progressively increases to amounts far above normal (19). T_4 administration in this state rapidly normalizes the pituitary content as well as the serum level of TSH (19).

In man the positive effect of thyroid hormone on TRH synthesis has been observed in a hypothyroid patient suffering from Hashimoto's thyroiditis and partial thyroid hormone resistance. T_4 substitution was followed by a paradoxical rise in TSH level, that could only be normalized by substitution of 150 μg T_4 plus 100 μg T_3 (20).

Yet, the levels of TRH in peripheral blood in man are raised in primary or secondary hypothyroidism and diminished in hyperthyroidism. The TRH level normalizes after therapy (21). Though the differences in the rates of removal of TRH in these conditions partly explain the different levels, the differences are too large to be the sole explanation.

In normal rats, T_4 treatment decreases the serum TSH level, but also the pituitary content (19), but the pituitary stores are quickly normalized after TRH injection (22). This favours the idea that the release of TRH is suppressed by raised thyroid hormone levels. It may be the reason for a normal or even higher hypothalamic TRH content in hyperthyroidism (11).

Pharmacological doses of glucocorticoids suppress the release of TRH. Glucocorticoid withdrawal may be used as a test of TRH reserve (23).

With advancing age, TRH production progressively decreases. So the "setpoint" for the thyroid hormone levels will decrease with age. Indeed, the T_4 levels and especially the T_3 levels decrease with age (24,25).

1.2.6 *Function of TRH*

TRH is accumulated by the pituitary because of specific binding to

membrane receptors, associated with adenylcyclase. TRH stimulates this adenylcyclase, leading to enhancement of the formation of cyclic AMP, which in turn eventually leads to TSH production and -release (1). Not only this sequence of events involved in the stimulation process follows the classic way of hormonal action; as documented for other hormones, TRH also reduces the number of its receptors when it is present in increasing amounts (26).

The release and synthesis of TSH are not controlled in the same way. The TSH synthesis stimulated by TRH is not inhibited by thyroid hormone in the rat, in contrast to its release (22).

TRH is not specific in its function of releasing hormone; prolactin is released from the pituitary as well and FSH is released in men but not in women (27).

As TRH is spread all over the brain, one might even wonder whether TRH has other functions than that of releasing hormone. TRH produces behavioural effects in experimental animals. When injected in those regions in the brain where TRH is thought to be produced, TRH leads to behaviour that enables to adapt to cold; shaking and vasoconstriction (28). On frog spinal cord motoneurones TRH has an excitatory transmitter effect (29). Whether TRH has psychoactive properties in man, is still controversial, especially regarding its antidepressant action (30,31,32).

1.2.7 *TRH analogues*

TRH analogues have been described, but most of them are of inferior potency compared to TRH (33), except for N(3im)-methyl-TRH which has about 8 times the potency of TRH (34,35) (see figure 1-2). Its affinity towards thyrotropic cell receptors is also 8-10 times higher than the TRH affinity (36). Methyl-TRH has been found in mammal brain (37), so it might constitute a "natural analogue" of TRH. It is cleared by serum enzymes about two times slower than TRH (38).

1.2.8 *TRH deficiency*

Hypothyroidism as a result of TRH deficiency has been described by many authors (23, 39-45). In most cases the deficiency is the result of a blockade or a disruption of the hypophyseal portal system by a trauma, operation or tumor. Some cases are thought to be caused by infection of the hypothalamus and few are probably inborn.

1.2.9 *TRH overproduction*

Hyperthyroidism caused by TRH overproduction has been proposed once (46). But, as discussed in an article presenting a similar case and reviewing other cases (17), TRH overproduction can hardly be looked upon as the sole reason for the hyperthyroidism, as long as the TSH can not be suppressed by T_3 overdosage. Selective pituitary resistance to thyroid hormone or inappropriately controlled secretion of TSH by pituitary micro-nodules constitute the best explanation (47-49), until TRH measurements prove the contrary.

1.3 THE PITUITARY

The pituitary is located beneath the cerebrum in the sella turcica. The posterior lobe, or neurohypophysis, secretes compounds as vasopressin and oxytocine. These compounds are formed in the paraventricular nucleus of the hypothalamus, and are transferred in a similar way as the releasing hormones, down to the endings of the axons located in the neurohypophysis.

The anterior lobe or adenohypophysis, consists of a variety of cell types, each producing and secreting different tropic hormones. The synthesis and release of these hormones are controlled by hypothalamic hormones with releasing or release-inhibiting potency.

One of the tropic hormones released by TRH is the glycoprotein TSH. TSH is produced in the pituitary thyrotropic cells; it is stored in small granules in these cells.

1.3.1 *TSH-production*

TSH is a glycoprotein with a molecular weight of about 28,000 containing about 15% carbohydrates. It is composed of two non-covalenty bound peptide chains, called α and β, each with a length of about 100 aminoacid residues.

The α-chain of TSH is identical or almost identical to the α-chains of LH and FSH. The β-chain of TSH differs from the β-chains of LH and FSH, though several regions of homology are present. Even in the α and β-chains regions of homology exist.

The free α and β-chains have little or no hormonal activity. After combination of a β-unit of one hormone with an α-unit of one of the other types, full hormonal activity with regard to the β-unit is restored. So the specificity of the hormonal activity is located on the β-unit, but the assemblage to an α-unit is necessary for its expression.

In the pituitary free α and β-chains, together with complete TSH, are found. The amount of α-chain is always higher than that of the β-chain. The release of free chains is governed by the same mechanism as the release of complete TSH (50,51).

Evidence has been presented that the α and β-units are formed separately and combined afterwards (51). The production of the β-unit, outnumbered by that of the α-unit, would be the limiting factor in the production of active hormone.

The α-chain contains two carbohydrate chains, the β-chain only one, all connected to aspartylic side chains. Carbohydrate chains are formed by chain lengthening. Molecule by molecule is attached during the passage of a completed peptide chain through the endoplasmic reticulum. The lack of precise control on the formation of the carbohydrate sidechains leads to differences in carbohydrate content. This microheterogenity, demonstrable by electrofocussing for instance, is not known to interfere with hormonal activity.

1.3.2 *Control of synthesis and release of TSH*

The synthesis and the release of TSH are stimulated by TRH. The dose response relationships of synthesis and release to stimulation

with TRH or analogues of TRH are different (33); they even might involve (partly) different pathways of activation, as in rats the TRH stimulated synthesis is not blocked by thyroid hormone (22).

A number of subsequent events is involved in the release of TSH. TRH binds to specific pituitary membrane receptors, leading to cAMP formation, responsible for the ultimate release of TSH.

The release of TSH is inhibited by thyroid hormone. Whether T_4 has some activity by itself in this respect or has to be converted to T_3 to be active, is still a matter of debate.

In the thyrotropic cells a very limited number of specific nuclear binding sites for T_3 is present (52). The inhibiting effect of T_3 on TSH release is protein synthesis dependent. The general view at the moment is, that binding of T_3 to its nuclear sites induces the synthesis of a specific protein, capable of inhibiting TSH release. The synthesis of this "blocking protein" can be prevented by actinomycin D (53). The necessity of de novo synthesis explains the lag fase of about one hour between T_3 injection and its blocking effect (54).

Apart from other influences on TSH release, which will be reported later on and which modulate rather than control, the opposing actions of TRH and thyroid hormone fundamentally control the TSH release. The constant stimulus by TRH is counter-balanced by constant inhibition by thyroid hormone, forming a sort of "thermostat", in which TRH is responsible for the "set point". Deviation from the equilibrium values at the "set point" of either TRH or thyroid hormone, is followed by a change in TSH release, until the equilibrium values are reached again.

Several features of this control system of TSH release will be discussed later, but one has to be mentioned here. TRH does not only stimulate TSH release and -production, but also the growth of thyrotropic cells. Long-standing overactivity as in hypothyroidism is accompanied by selective hypertrophy and hyperplasia of the thyrotropic cells, leading to pituitary enlargement (55). In this situation thyroid hormone substitution of course decreases the TSH release (56), but when production and release of TSH do not become balanced, progression of the pituitary enlargement may cause

symptoms, analogous to the complications associated with pituitary tumors (57). This situation might be equivalent to the imbalance observed after TRH injection in rats treated with T_4, were TSH synthesis resumes although the release is inhibited (22).

1.3.3 *TSH response to TRH*

In normal man the response of the thyrotropic cells to intravenous synthetic TRH can be followed by measurement of serum TSH levels. Preinjection levels of TSH are low. The upper limit of the normal range differs from laboratory to laboratory, but in general a level of about 7 μU/ml is reported.

After rapid i.v. injection of TRH, peak levels of TSH are observed after 15 to 30 minutes, which decline to within normal limits after one or two hours. The mean maximum rise varies with age from 10 to 20 μU/ml. A significant TSH response is observed after injection of as little as 10 μg of TRH. The maximum increment of TSH is related to the TRH dose in a roughly linear way up to doses of 200 to 400 μg (58-60). One report indicated the maximal response in normals to occur with a dose of 30 μg TRH (61). The individual variation of the response is large, but the intra-individual variation is much smaller (59). Nowadays most centers use a 200 μg dose of TRH for normal practice.

The response of serum TSH to methyl-TRH, though following the same pattern in time, seems to be substantially higher compared to TRH (34). A 100 μg dose of methyl-TRH seems to bring about a maximal response.

Prolonged infusion of TRH leads to sustained release of TSH (45,62). This prolonged elevation of TSH makes it possible to follow the thyroid hormone response to endogenous TSH (45,63).

1.3.4 *Influences on basal and TRH stimulated TSH*

a. *Influence of thyroid hormone*

The most profound influence on TSH release is exerted by the level

of thyroid hormone. Serum TSH is undetectable or very low in hyperthyroidism. It is raised in primary hypothyroidism and reaches very high values after TRH stimulation in this condition. Even within normals, there is a negative relation between serum T_4 and TSH response (64), as well as a positive relation between basal TSH and TRH stimulated TSH (64). Most authors do not observe a relation in normals between basal T_3 and basal TSH or TRH-stimulated TSH. Within an individual, however, basal levels of T_3 and TSH are even positively related (61). The pituitary is very sensitive to small changes in thyroid hormone production (65), as will be discussed later.

b. *Influence of daily rhythm*

The serum TSH levels shows a circadian pattern, being higher during the night than during daytime (66-69). Strong evidence has been obtained that this pattern is controlled by the hypothalamus and does not result from circadian changes of responsiveness of the pituitary (70,71). Endogenous depression is associated with a disturbed circadian pattern (72).

The TSH response to TRH seems to be higher in the evening than in the morning (73). In a smaller group of individuals this difference was not found (74). The relation between the preinjection level of TSH and its maximum value after TRH stimulation is present throughout the day (73).

c. *Other influences*

Several authors observed a higher response to TRH in women compared to men, others did not. As the groups differed with regard e.g. to age, weight and day of menstrual cycle, no definite conclusions can be drawn.

Oral contraceptives may influence the daily TSH pattern by enhancement of the TRH burst during the night, resulting in a pattern with a higher amplitude (71). This fits the observation that the TSH response to TRH is higher during oral contraceptive

medication (75), though in an other report the basal TSH and TRH stimulated TSH is claimed to be lower during contraceptive medication of the combined type (76). Estrogen medication during five days in postmenopausal women does not change the TSH response to TRH (77).

Data on the role of glucocorticoids are more consistent. Higher doses, given for a short period, suppress TSH release, presumably through hypothalamic action (23,46,78-82).

The role in vivo of dopamine and serotonin is still obscure. Infusion of dopamine strongly inhibits the TSH release leading to suppressed basal and TRH stimulated TSH levels (83). Long-term medication with L-DOPA has the same effect (84). Acute effects of L-DOPA are only consistently found in long-standing hypothyroidism, where basal levels of TSH decrease (85,86). This means that "exogenous dopamine action" is mainly confined to an inhibiting effect upon the TSH release.

Adrenergic blockade as well as epinephrine administration were once reported not to affect the TSH response to TRH (87), but in an other article a reduced response was found after phentolamine treatment (88). Apomorphine, considered as a direct dopamine receptor stimulator, administered s.c. in a dose of 0.75 mg, did not affect the basal or TRH stimulated TSH levels (89,90).

Cyproheptadine, a serotonin antagonist with antihistaminic, anticholinergic and antidopaminergic properties, reduced the TSH response to TRH (91). Methergoline, a specific blocker of central serotonin receptors, did not affect the TSH release (91).

The data on the effect of growth hormone release inhibiting hormone (= somatostatin) are again more consistent. Infusion of this hormone blocks the TSH response to TRH in a dose dependent way (92) and is capable of preventing the nightly TSH surge (93,94). Its action is short-lived, soon after stopping the infusion the nightly TSH surge resumes. In this respect the observation is interesting that growth hormone treatment leads to reversible secondary hypothyroidism in selective growth hormone deficiency and to blunting of the TSH response in growth hormone deficiency

combined with tertiary hypothyroidism (95). The authors postulate this phenomenon to be mediated by somatostatin secretion in response to pulse doses of growth hormone.

1.3.5 *Seasonal variation of TSH secretion*

In adult man there is no difference in basal TSH in cold wintertime compared to summer (96). The acute rise of TSH after birth, however, is higher during winter compared to summer (97). This difference was found not to be due to differences in room temperature. After 4 hours the "winter" TSH levels declined to the "summer" levels.

1.3.6 *Variations of TSH secretion with age*

Little is known about at which stage during gestation TSH production starts. TSH is not detectable in amniotic fluid (98). The TSH level in cord serum is above the normal upper adult level (97,99). There is an acute rise of TSH to levels of about 100 μU/ml 30 minutes after birth (97), which, after an initial sharp fall, further slowly decline to mean levels comparable to cord serum values after two or three days (99). Within 6 weeks TSH levels are within the normal adult range (99). In children and adolescents, when challenged with weight adapted TRH doses, the TSH response is comparable to the response occurring in young adults (44). The response of TSH in adults declines with age (60). The response in persons over 60 years is about half of the response seen in persons of about 30 years.

1.3.7 *TSH deficiency (secondary hypothyroidism)*

TSH deficiency has been known to occur, partial or complete, in pituitary disease. It is found in Sheehan's syndrome, with intrasellar tumors and after hypophysectomy. There is, in its pure form, no reaction of the low TSH levels to TRH injection. Often in these cases the hypothyroidism originates from a combined failure of the

hypothalamus and the pituitary. Mostly TSH deficiency is accompanied by deficiency of other pituitary hormones.

Isolated TSH deficiency has been observed in a familial form (100) as well as in an acquired form (101,102). Though the response of TSH to TRH does not at all or hardly exceed the limit of detectability, minimal amounts of TSH are released, as judged from the enhanced release of thyroidal iodine (101).

1.3.8 *TSH overproduction*

The possibilities of TSH overproduction have already been mentioned in the context of TRH overproduction. Hyperthyroidism of this type seems to be associated mostly with chromophobe adenoma or other pituitary tumors. Out of a group of 62 pituitary tumor patients, two had hyperthyroidism due to inappropriate TSH secretion (103). In some cases there is no TSH reaction to TRH (46,48) and in most cases there is hardly any suppression by T_3 administration (46,48,103,104). Somatostatin depressed the TSH level (48) in one patient studied. Mostly methimazole medication was followed by a rise in TSH (46,48,105,106). Dexamethasone was able to suppress TSH in the one patient studied (46), but here no tumor had been detected.

1.3.9 *The TRH-test for clinical use*

Measurement of serum TSH before and after stimulation with TRH provides nowadays the most sensitive diagnostic tool for abnormalities in the hypothalamus-pituitary-thyroid axis (23,58,59,60,107,108).

Primary thyroid failure is characterized by a high basal level of TSH and a huge response of TSH to TRH. Even impending failure, called preclinical hypothyroidism, can be diagnosed. In this condition the serum thyroxine level is at the lower limit of normal, that of triiodothyronine normal, whereas the TSH level is above the upper limit of normal already. This situation is often encountered after ^{131}I treatment for hyperthyroidism (109) and may be stable for several

years (109). This also applies to the situation after subtotal thyroidectomy (110) and idiopathic euthyroid goiter (111). TRH stimulation is followed by massive TSH secretion.

In hypothyroidism the amplitude of the response depends on the length of the interval during which hypothyroidism developed. In long-standing preclinical hypothyroidism the basal TSH level is just above normal limits and reaches values above 100 μU/ml after TRH stimulation. Shortly after surgery or ^{131}I treatment resulting in hypothyroidism, basal TSH levels are in the order of 40 μU/ml and rise only to values of 60-70 μU/ml upon TRH challenge. In long-standing hypothyroidism basal values between 30 and 150 μU/ml are found, which can rise to levels of 400 to 500 μU/ml upon stimulation.

Hyperthyroidism can be precisely diagnosed, as the pituitary is very sensitive to the raised thyroid hormone levels. Basal levels are low or even undetectable and do not react to TRH, even when large doses are given of e.g. 1,000 μg. Even preclinical hyperthyroidism, due to e.g. autonomous nodules, with thyroid hormone levels in the normal range, can be diagnosed by a negative TRH-test (112).

It is easy to understand that the TRH test and the T_3 suppression test do not always show parallelism. In general a negative TRH test is equivalent to a negative T_3 suppression test; but in case the autonomous part of the thyroid does secrete less hormone than the daily need, the TRH test will be normal in spite of a negative suppression test (113). Because of the sensitivity of the pituitary for thyroid hormone one must be aware of changes in the TRH test results unrelated to thyroid abnormalities. Even the small displacement of thyroid hormone from the serum binding proteins by competing drugs interferes with the results for some weeks at the start of the medication (114).

Small decreases of thyroid hormone levels due to iodide administration significantly enhance the TSH response (65) as well as the basal TSH level (115). These changes are understandable, because as little as 6 μg T_3 p.o. per day is able to change a "hypothyroid TRH test" into a "hyperthyroid TRH test" (116).

The TRH test is of some help in the diagnosis of hypothalamic and

pituitary abnormalities. Mostly hypothyroidism resulting from idiopathic hypopituitarism can be considered as to be due to hypothalamic disorders as far as the TSH response to TRH is not absent. In these cases sometimes the peak response is delayed and exaggerated (44,117,118).

A diminished TSH response to TRH can be found in hypopituitarism due to pituitary tumors (40,117,118). However, euthyroidism is not rare in cases of pituitary tumor or Sheehan's syndrome, with a virtually absent or limited TSH response (23,40, 118). A blunted TSH response is also found in Klinefelter's syndrome (119), probably related to the long standing hypergonadotropic state.

Suprasellar and partially suprasellar tumors show TSH responses that vary between "pure hypothalamic" and "pure pituitaric" hypothyroidism. The TRH test is of little help in the differentiation between these tumors. Whether the combination of the TRH test and the glucocorticoid withdrawal test might provide a better discriminative tool seems doubtful (23).

Sometimes in hypothalamic hypothyroidism the basal level of TSH is just above the normal upper limit. It has been suggested that the TSH secreted in this condition has less biological potency (39). However, as this situation is found almost exclusively in young people with idiopathic hypothyroidism of moderate degree (39), one must consider the possibility of lifelong thyroid alterations resulting from neonatal hypothyroidism as found in the rat (120), superimposed on the TRH deficiency.

In older patients a restricted TSH reserve and a partial thyroid failure can be the cause of hypothyroidism. In this situation the basal levels of TSH are elevated, but do not respond to TRH (121).

Acute and chronic stress is followed by a number of changes in the hypothalamus-pituitary-thyroid axis. One of them is the change to a state comparable to hypothalamic hypothyroidism (122). The TRH test in anorexia nervosa points to similar abnormalities (123,124).

1.3.10 *TSH-action*

TSH is specifically bound to thyroid membrane receptors. After binding, TSH stimulates a number of thyroidal processes. Most of them are directly related to stimulation of cAMP formation. The dose response relationships of the processes are different. The active accumulation of iodide fluctuates with changes of TSH in the lower range, whereas cell multiplication is only enhanced at higher TSH levels.

TSH also stimulates the reabsorption of iodide in the kidney. As TSH shows a diurnal pattern, iodide excretion changes as well. In experimental animals the effect of endogenous TSH, exogenous TSH and exogenous TRH has been studied (125-127). The results show that the diurnal pattern of iodide excretion is not related to changes in filtration rate, but only to the TSH level.

As in other hormonal systems, TSH stimulation of the thyroid is followed by refractoriness (128). This refractoriness is specific; the stimulation by other hormones as e.g. prostaglandins is not influenced. Protein synthesis is not needed for this phenomenon. It is assumed that the effect is due to a decline in TSH receptor sites.

1.3.11 *TSH-like activity*

Hyperthyroidism or thyroid autonomy seen in Graves' disease is associated with immunoglobulins present in the serum, possessing TSH-like activity. The exact role of immunoglobulins with thyroid stimulating properties in human thyroid pathology is still unsolved (129-134). For the immunological processes involved is referred to a recent review (135).

With regards to TSH receptors, some results are interesting. The immunoglobulins with thyroid stimulating activity are capable of competition with TSH for its membrane receptors (131,134). This does not mean that the immunoglobulins react with the receptor as such. Binding of the immunoglobulins may result in steric hindrance of the TSH binding. Antimicrosomal antibodies (without thyroid stimulating properties) are capable of preventing a stimulus by TSH

or immunoglobulins with stimulating activity, probably through prevention of binding of these stimulators to the membrane (136). So, in an autoimmune process, circulating immunoglobulins may bind to thyroid cell membranes in such a way that the TSH receptor sites become blocked or hidden. For one group of immunoglobulins the binding means stimulation as well and hyperthyroidism with or without a goiter may develop. For the other group the binding results only in a blockade of the TSH receptor.

Immunoglobulins stimulating human thyroid cells can bind to animal thyroid cells without stimulating them. Immunoglobulins that bind to human thyroid cells, but do not stimulate them, may activate animal thyroid cells (135).

In normal pregnancy a chorionic thyrotropin is secreted by the placenta. Overall thyroid function is not affected and the levels of the free thyroid hormones stay within normal limits.

In molar pregnancy and other trophoblastic tumors a high molecular weight substance, not related to pituitary TSH, is present (137). This material has TSH-like activity and clear hypermetabolism or even thyrotoxicosis is the result (138,139). It may be identical to the material found in commercial urinary HCG preparations (140), which might indicate that also in normal pregnancy it is produced in minor amounts. Probably this substance is abnormal HCG that is different from normal HCG in its carbohydrate content (138), which may be the reason that the TSH-like activity does not correlate to the total HCG level normally, in contrast to the good correlation with the serum HCG level in molar pregnancy (139).

The thyroid is also stimulated by prostaglandins (141,142), but this is probably of minor importance in the overall control. There also is some stimulation of the thyroid via the neural route, but in the human situation this plays a small role (143).

1.3.12 *Fate of TSH*

Though the exact way of the elimination of TSH is unknown, the clearance rate is mainly dependent on kidney function and basal

metabolic rate. Before removal from the bloodstream TSH is not dissociated in its free α and β-units (50,51). In normals the metabolic clearance rate is about 25 ml/minute per m^2 surface. The clearance rate is strongly positively correlated to the creatinine clearance, the T_4 level and the T_3 level (56). The daily production of TSH amounts to 104 ± 41 mU per day (mean $\pm$SD) (56) in normals. This value may be too low, as it is based on TSH levels measured during daytime.

1.4 THE THYROID

The thyroid gland normally consists of two lobes connected by a bridge and is situated at the cartilage of the larynx. Two types of cells are present in the thyroid. One type, the C-cell, produces calcitonin. The other type secretes thyroid hormones. These cells surround in a monolayer a lumen filled with colloid, forming together a follicle. Several of these follicles form a lobule, that is supplied by one arterial vessel. The follicular cells contain a large amount of rough endoplasmic reticulum and a large Golgi apparatus. The colloid consists almost exclusively of a glycoprotein formed by the follicular cells: thyroglobulin. TSH is needed for the proper differentiation of the follicular cells. In in vitro cultures thyroid cells grow in layers; only after addition of TSH follicles are formed. TSH activity enhances cell multiplication. Only sustained stimulation by high TSH levels leads to goiter formation. Other stimulators than TSH have the same effect.

1.4.1 *Iodide accumulation*

The thyroid can concentrate iodide by means of an active process. This accumulation process is coupled to an ATP-ase present in the cell membrane. Iodide is transported into the cell against an electrical (50 mV) and concentration gradient. Normally only 60 μg of iodide is outside the thyroid and about 6 mg of iodine inside.

The accumulating system, the iodide trap, is stimulated by TSH action. The activation proceeds through c-AMP. The activity of the trap is governed by changes of TSH at low levels.

The system is not specific. Anions resembling iodide are recognized as iodide and may compete for transport. Perchlorate, pertechnetate and thiocyanate are well known in this respect.

The system in the kidney responsible for iodide reabsorption shows similarities to the thyroidal iodide trap, as e.g. thiocyanate competitively inhibits reabsorption in the kidney.

1.4.2 *Iodide organification*

After entrance, the iodide is very quickly built into proteins. A microsomal enzymatic system is involved in this process. Iodide, in the presence of hydrogenperoxyde, reacts with a sulphhydryl group of the active center of the enzyme to form a S-I group (see figure 1-3).

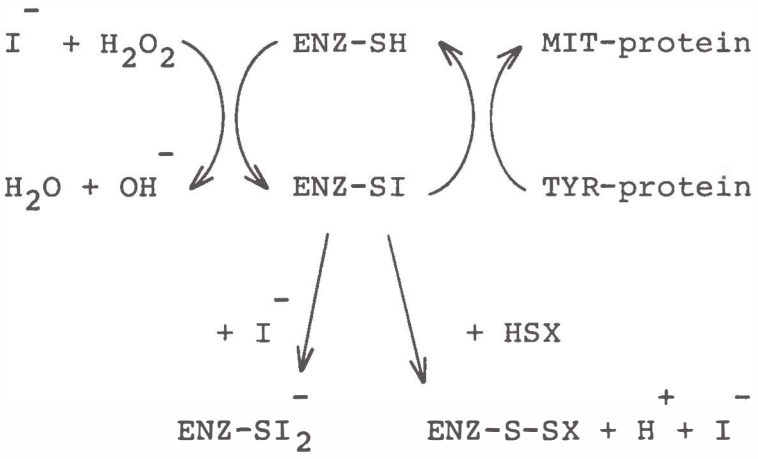


Figure 1-3. The iodinating system in the thyroid.
ENZ = enzyme; HSX = thiouracil

The hydrogenperoxyde is formed with the aid of NADPH, which is generated in the pentosephosphate cycle. The iodide activated in the form of the S-I group is than substituted to aminoacid side chains of available proteins. Especially the tyrosyl groups are apt to substitution, but also histidyl groups are iodinated (144). As thyroglobulin is the most abundant, almost all accumulated iodide is substituted to thyroglobulin. However, other proteins as e.g. albumin are iodinated as well.

In those cases where thyroglobulin synthesis is abnormal, as in congenital defects and thyroid tumors, the relative amount of these other iodinated proteins enhances (145,146).

The iodination starts already in the endoplasmic reticulum but is performed mainly in the apical vesicles, especially near the apical cell border during the process of emptying of the vesicles into the colloid. Some iodination occurs also in the colloid.

The active S-I group of the iodinating system can be inactivated by several compounds. With an iodide excess a very stable inactive S-I_2 group is formed by addition (see figure 1-3). Other compounds can poison the enzyme as well. Thiouracyl compounds react with the active S-I group by substitution of the iodine (see figure 1-3). The enzyme poisoning can be studied by a perchlorate discharge test (147).

1.4.3 *Thyroglobulin*

Thyroglobulin is a glycoprotein with a molecular weight of about 660,000. Normal thyroglobulin contains about 0.5% iodine. Thyroglobulin is normally composed of two identical subunits. The monomers of thyroglobulin are composed of two covalently bound peptide chains (148). These chains are formed on the ribosomes of the endoplasmic reticulum. After completion of the peptide chains and combination of both types of chains, the monomer is transported through the lumen of the endoplasmic reticulum to the Golgi apparatus. During this passage the carbohydrate chains are formed by a chain lengthening process, attaching monosaccharide by monosaccharide. In the Golgi apparatus the thyroglobulin, already iodinated to a minor degree, is packed into small vesicles, the apical vesicles, which move towards the apical cell border. The vesicles fuse with the cell membrane, emptying their content into the colloid. During the transport in the vesicles and the fusion process most of the iodination occurs.

1.4.4 *Formation of thyroid hormones*

Upon iodination, the thyroglobulin molecule undergoes conformational changes; the first iodinated tyrosyl residues move inwards, exposing other tyrosyl side chains to be iodinated. Finally some iodine has been incorporated in the molecule at sites not involved in the process of hormone formation, but the majority of the iodine is present in tyrosyl residues pointing out of the molecule.

As one or two iodine atoms per tyrosyl residue can be incorporated, monoiodotyrosyl (MIT) as well as diiodotyrosyl (DIT) residues are present at the outside of the molecule. These iodinated tyrosyl residues are highly reactive.

To a iodotyrosyl group another iodotyrosyl group can be coupled, forming a iodothyronyl group. Whether this second iodotyrosyl group stems froms a second thyroglobulin molecule or from free iodotyrosine or an analogue of iodotyrosine, is still unclear. Maybe both routes are used. Most authors believe that an enzyme, the coupling enzyme, is involved in the coupling reaction.

Combination of monoiodotyrosyl and diiodotyrosyl groups leads to different thyronyl groups. As normally diiodotyrosyl- outnumber monoiodotyrosyl groups, tetraiodothyronyl groups are abundant, but triiodothyronyl groups are present in fair amounts too. Only minor amounts of other combinations as e.g.reversed triiodothyronyl (rT_3) and diiodothyronyl (T'_2) groups are formed (see figure 1-4). The

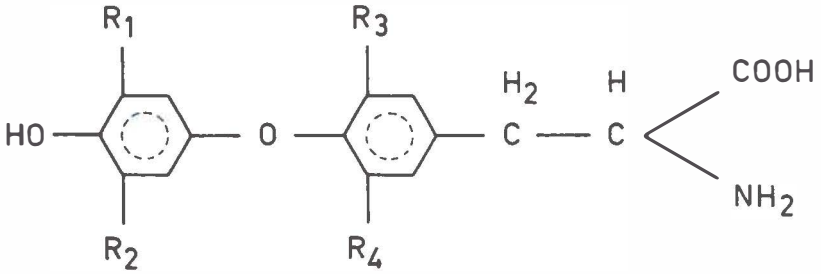


Figure 1-4. Structure of iodothyronines.
T_4: $R_1=R_2=R_3=R_4 = I$
T_3: $R_1=R_3=R_4 = I$ and $R_2 = H$
rT_3: $R_1=R_2=R_3= I$ and $R_4 = H$
T'_2: $R_1=R_3 = I$ and $R_2=R_4 = H$

molar secretion rates of T_4, T_3 and rT_3 in the normal human have been estimated to be 85:9:1 (149). This is close to the relative molar proportions found in normal thyroglobulin (150) (T_4:T_3 = 9.3 and T_4:rT_3 = 75.5).

1.4.5 *Thyroid hormone secretion*

Upon stimulation by TSH, colloid droplets are formed within the cell by pinocytosis. On the apical cell membrane a circular protrusion is formed, which eventually closes, thereby surrounding a part of the colloid and forming a colloid droplet. Colloid droplets are much larger than apical vesicles. The colloid droplets contain colloid from the vicinity of the apical membrane, that means thyroglobulin that has been formed recently. So: last come, first served.

The colloid droplet moves to the opposite side of the cell and fuses on this route with a lysosome. The enzymes of the lysosome hydrolyze the thyroglobulin, liberating the aminoacids and amongst them MIT, DIT, T_3 and T_4.

Then T_3 and T_4 leave the cell and reach the general circulation. Most of the MIT and DIT is deiodinated by a cytoplasmic enzyme, tyrosine dehalogenase. Part of the iodide thus formed leaves the cell, but most of it is reused in the iodination of thyroglobulin. This explains the 10 month period of euthyroidism after iodine repletion was stopped in a patient with a trapping defect (151). A dehalogenase defect, or inhibition of this enzyme (152), is characterized by loss of MIT and DIT in the urine, leading to iodine deficiency and hypothyroidism (153).

Normally, not all of the thyroglobulin is completely hydrolyzed. Even completely intact thyroglobulin leaves the cell and thus forms a normal constituent in the blood.

The process of hormone secretion is blocked by excess of iodide and by lithium (154). Which step in the whole process is inhibited is not certain, probably it is the colloid droplet formation (141,155). The inhibition is overcome after prolonged excess or prolonged lithium medication, probably due to iodide pooling in the thyroid.

1.4.6 *Control of thyroid hormone synthesis*

The most important regulators of thyroid hormone synthesis are the availability to the thyroid of iodine and TSH action. Chronic iodine deficiency, caused by a low iodine intake or by a goitrogenic blockade of the iodide trap, leads to a deficient hormone synthesis.

This in turn stimulates TSH secretion in an effort to enhance the iodide accumulation (156,157). In this situation the daily uptake of iodide and the intrathyroidal turnover of thyroglobulin get out of balance. The degree of iodination of thyroglobulin decreases, leading to a relative rise of the T_3/T_4 ratio in thyroglobulin (158) and in the serum (153,157). In this way, an attempt is made to match the iodine deficiency by the preferential production of the more potent, but less iodine containing T_3.

So with an increasing iodine amount per glandular tissue weight, the amount of both T_3 and T_4 per tissue weight increases, but in such a way, that the T_3/T_4 ratio drops (159).

A high T_3/T_4 ratio is also encountered in e.g. Hashimoto's thyroiditis (153,160) and the trapping defect (151). Even parts of the thyroid may show a relative iodine deficiency, though the total uptake of the thyroid is normal or even enlarged. In these cases T_3-hyperthyroidism may develop (161,162); Graves' disease in an iodine deficient area may also express as T_3 thyrotoxicosis (163).

In some cases thyroid autonomy remains hidden because of a limited iodide supply. A sudden rise of iodide availability, due to for instance an iodine containing contrast medium, may in these euthyroid cases rapidly provoke hyperthyroidism (Jod-Basedow) (161,162,164).

Injection of TSH results only in a moderate rise of the T_3/T_4 ratio in thyroglobulin as long as the availability of iodine is normal or high (158).

A partial inhibition of the iodinating enzyme leads to poorly iodinated thyroglobulin with a high T_3/T_4 ratio. This resembles an iodination defect (165). Excess of iodine blocks the hormone release in normals, eventually leading to a high T_3/T_4 ratio due to TSH stimulation (115).

The thyroid is to some extent self regulating. Day to day fluctuations of absolute iodine uptake due to intake variation, are not "translated" in day to day fluctuations of thyroid hormone secretion, because of the storage of thyroglobulin. Day to day variations in the degree of iodination of newly formed thyroglobulin - and thus in the T_3/T_4 ratio - are damped because of the mixing during storage. Probably, once in a while the intake during one day largely exceeds the normal daily intake, as indicated by the negative iodine balance commonly found in normals (166).

Gradual long term changes in the intake within the normal range are not regulated by the thyroid itself. Ultimately a small average increase in iodine intake is followed by a new equilibrium with a slightly lower TSH. The iodide trap, which is highly sensitive to small changes of TSH, is probably the most sensitive parameter in this respect. In this situation the uptake percentage may drop, leading to the same value of the absolute iodine uptake as before. This phenomenon urges one to check the local "normal values" for the uptake percentage periodically (167).

The sequential changes in the complete control system during iodine repletion have been documented in one patient (151). Especially preferential production of T_3 in the initial phase is impressive. As this case showed a congenital trapping defect the changes in iodine uptake were probably slow, thereby lengthening the whole sequence of changes and preventing an overshoot. Prolonged administration of an iodide excess to non-iodine-deficient normals is followed by a blockade of the hormonal secretion, resulting in a decrease of serum T_3 and T_4, until the TSH level rises to enhance the T_3/T_4 ratio (115,168). Longterm iodide medication may lead to reversible hypothyroidism (168), probably in those cases with a restricted thyroid reserve.

1.4.7 *Thyroid abnormalities*

a. *Enzyme defects*

As several thyroidal enzyme systems are involved in the formation

of thyroid hormones, defects in one of them, mostly inborn, lead to abnormal or deficient hormone synthesis. As far as the defect can not be overcome by enhanced TSH stimulation, severe hypothyroidism, even leading to cretinism, may develop.

Some of the defects are:

— defective thyroglobulin synthesis (169-173), which may not be one entity, as it is sometimes present together with a dehalogenase defect (169) or chromosomal aberrations (171);
— defective iodide trapping (151,174);
— defective iodinating system (165);
— defective dehalogenase (169,175,176);
— defective hydrolysis of thyroglobulin (145).

The defects sometimes are partial. In some of the defects the thyroid contains and secretes large amounts of iodinated albumin and prealbumin. When the thyroglobulin synthesis is practically zero, the organification of iodine may become defective, despite normal iodinating enzyme activity (173).

b. *Immune diseases*

As already noted, the thyroid may become diffusely enlarged and hyperactive due to stimulation by circulating immunoglobulins (Graves' disease). The aetiology is not resolved yet (129,177).

In cases of thyroiditis the immune system is also involved. There are several forms of thyroiditis and the nomenclature is confusing (178). In most cases the thyroiditis is chronical and lymphocytic, resulting from an autoimmune disease. In some countries the incidence of this type of thyroiditis is high, but symptomless in the majority of cases (179-181) and even reversible, as judged by antibody measurement (181). High titers of autoantibodies against thyroglobulin are mostly present. Generally, subclinical hypothyroidism is present, judged from the slight elevation of the TSH level (179), but frank hypothyroidism may develop (179,181).

In other types of thyroiditis, described as acute, subacute or chronical, the immune system seems to be involved only secondarily. In these cases the titers of thyroid antibodies are low in general. In the

initial phase of the subacute thyroiditis of the De Quervain's type, hyperthyroidism (182,183) or even thyrotoxicosis is present (184), though the iodine uptake is low. In most cases the disease evolves via a short period of hyperthyroidism into euthyroidism after 2-6 weeks. Normally, the differential diagnosis is not difficult, but one should be alert (185).

Some cases of hypothyroidism due to autoimmune thyroiditis have been described, which developed thyrotoxicosis some years after the first diagnosis and subsequent substitution (186). It is known that in Graves' disease - whether treated or not - eventually hypothyroidism may develop. The report of Graves' disease in one and Hashimoto's thyroiditis in the other of monozygous twins (187) may support the concept of a common genetic aetiologic factor in the pathogenesis of the two autoimmune diseases.

c. *Noduli*

Sometimes one or more nodules can be noticed in the thyroid upon palpation. Only a limited number of cases is found however by physical examination. Post mortem macroscopic inspection of 821 thyroids of euthyroid patients, in whom palpation had revealed no nodules, showed that 50% of them contained nodules. The ratio of uni- and multinodularity was 1 to 3 (188). In 35% of the nodular thyroids the diameter of one or more nodules was more than 2 cm. The nodularity increased in frequency with age: in the first years of life about 5%, enhancing to 50% at the age of 50 and to 100% at ages over 90. At all ages the incidence of nodularity was 10 to 20% higher in females than in males.

Histopathological examination showed that the most common nodule was the non-neoplastic involutional nodule ("colloid adenoma" or "adenomatous nodule"), found in two-thirds of the nodular thyroids. The second most common was the true adenoma, occurring in about half of the nodular glands. Malignant neoplasms were also present in this material: 15 cases of low grade primary "occult carcinoma" and a small focus of anaplastic carcinoma in 2

cases. Though the incidence of thyroid nodules is high, the aetiology is still obscure.

Some nodules can be visualized by scintigraphy using radioactive iodine isotopes or radioactive pertechnetate. In this way the nodules can be classified as warm, hot or cool, according to their accumulation of radioactivity compared to that of the adjacent parts of the gland (189). Hot regions almost always turn out to be autonomous adenomas, though sometimes they consist of functional tissue in a thyroid of which the rest has been destroyed by Hashimoto's disease (190).

1.4.8 *TSH-action on the thyroid*

a. *TSH-action on iodide accumulation*

Injection of bovine TSH stimulates the trapping of iodide. The uptake percentage of a tracer dose of iodide rises (191), indicating an enhancement of the total iodide uptake. When the trapping system is already stimulated to its maximum by very high levels of endogenous TSH, no further increment is seen (191).

Injection of TSH stimulates the iodide uptake of normal thyroid tissue and autonomous tissue to the same degree, as long as the nodule is small enough and does not suppress the rest of the thyroid (192). However, when a hot nodule produces hyperthyroidism, TSH is suppressed. Therefore the normal tissue is inactivated and does not accumulate iodide (192). In a group of patients it could be shown that hot nodules accumulated on the average ten times more iodide than the rest of the thyroid. Administration of bovine TSH stimulated both nodules and the remaining tissue, with factors of 3 and 15 respectively. This means that under exogenous stimulation the nodules still cleared 2 times more iodide than the rest of the thyroid. As a consequence of the accelerated uptake, the plasma iodide concentration falls to half of its initial value. So, the rise in absolute iodide uptake rate is only half of the rise in thyroidal iodide clearance (192). This means that "autonomous nodules" are very susceptible to TSH action. It has been suggested (193) that focal hypersensitivity to

TSH is responsible for the occurrence of hot nodules and the growth of them. The level of endogenous TSH, ultimately suppressed to its minimum (194-197), still activates the nodule; T_3 medication does not change this (193,194,196,197). As the turnover of iodine is higher in nodules compared with normal tissue, a relative iodine deficiency results, leading to a higher T_3/T_4 ratio (195,198).

b. *In vitro TSH-action*

TSH activates the adenylcyclase and the protein kinase of thyroid tissue. It also enhances glucose oxidation and phospholipid production. Thyroid tissue from Graves' disease patients is not different from normal thyroid tissue in its reaction to TSH in this respect (199,200). However, tissue from hot nodules is more sensitive to TSH-action than adjacent normal tissue, regarding the activation of adenylcyclase and glucose oxidation (193).

c. *TSH-action on hormone secretion*

Upon stimulation with exogenous TSH the thyroid secretes an enhanced amount of T_4 as well as T_3. The response is sigmoidal to log dose, with a half maximal response at 7.7 mU bovine TSH/kg, reaching the plateau value of the response at about 100 mU/kg (201,202). The increases in serum T_4 and T_3 are about linear during at least four hours at all doses, whereas the weight ratio of the increases is constant at all doses and all intervals in normals, and approximates 20 (201,202). As the fractional response of serum T_3 is higher than that of T_4, measurement of T_3 is the most convenient way to judge a response of hormone secretion. There is no sex difference and no age dependency of the response (201,202). This means that the lowered hormone levels at advanced age do not result from a reduced sensitivity to TSH.

The reaction to endogenous TSH can be followed after TRH administration (45,61,63,107,203). The rise of T_3 is strongly positively related to the rise of endogenous TSH in an individual (61). The response of T_3 to rapid TRH injection seems to be diminished in TRH deficiency (107) and in other hypothalamic-pituitary disorders (203), when judged in relation to the TSH response. Atrophy of the thyroid and biologically less potent TSH are suggested both as the cause of this phenomenon. Prolonged infusion of TRH stimulated TSH secretion long enough to provoke readily measurable rises in the level of T_4 (45).

d. *Influences on the response to TSH*

T_3 medication, of 120 μg/d in four divided doses for two days, suppresses the T_4 level in normals. In this situation a single injection of 5U bovine TSH is followed by a smaller, absolute as well as fractional, response of the serum T_4 level (204). These results were interpreted as evidence for a short loop feed back mechanism within the thyroid (204). The possibility that the state of activity of the thyroid is the cause of this difference, seems to be overlooked. After T_3 medication the TSH level will be suppressed and the thyroid will be in a state of inactivity. TSH injection in this situation will be followed by an interval during which the thyroid is brought to the same state of activity as before T_3 suppression. By that time a substantial proportion of the injected TSH will be cleared and the T_4 response will be lower, as the injected dose is below the plateau value of the maximum response (202).

Short-term dexamethasone administration lowers the TSH release in normals. This lowering of the setpoint is followed by a decline in the serum T_4 level and especially the T_3 level (205,206). A single injection of bovine TSH in this situation is followed by the same absolute rises of the serum T_4 and T_3 levels as before dexamethasone treatment (205). This situation resembles the state of the control system as a whole at advanced age.

1.5.1 *Binding to serum proteins*

After T_4 and T_3 have been transported into the bloodstream, both hormones are almost completely (99.95 and 99.5% respectively) noncovalently bound to serum proteins. An α-globulin, thyroxine binding globulin (TBG), has a high affinity binding site for T_4 and T_3, with a ratio of affinities of about 10. A prealbumin, thyroxine binding prealbumin (TBPA), has different binding sites for T_4 and T_3 (207), both with lower affinities than those of TBG (208). TBPA also binds the retinol binding protein - vitamin A - complex, but at a different site.

Albumin shows a low affinity to T_4 and T_3. Because of its abundance, still a relative large amount of T_4 and T_3 is bound by this protein. Minimal amounts are bound by other proteins, such as low-density lipoproteins and high-density lipoprotein (209).

In normal serum the distribution of T_4 and T_3 over the major binding proteins is about: TBG 63% and 71%; TBPA 17% and 8%; albumin 8% and 12% (207). Changes in the levels of total T_4 or T_3 alter these distributions. As more high affinity sites, especially of TBG, become occupied with rising hormone levels, measurement of these binding sites serves as a thyroid function test. When the unoccupied binding sites are made competitive for radioactive hormone with a second binder as e.g. red blood cells, charcoal or resin, the uptake by the second binder becomes inversely related to the amount of unoccupied high affinity binding sites.

The free hormones are the metabolically active fractions. However, the determination of the free hormones is cumbersome (210,211). The combination of the measured total level of the hormones and the measured resin uptake serves as a good substitute (212-214). When using tracer T_3 in the resin uptake test, a correction for TBPA binding must be made in the computed free T_4 amount (208).

Normal amounts of TBG and TBPA are, expressed as their binding capacity of T_4, about 15 to 30 μg/100 ml and 100-240 μg/100 ml respectively (215-218). On a weight basis the level of TBG is about 700 to 1,200 μg/100 ml in young adult normals (219,220). Higher levels for normals were published earlier (221) but the method employed was less direct.

1.5.2 *Changes in TBG and TBPA content*

a. *Congenital changes*

Familial TBG deficiency is known (215,222,223). The deficiency is linked to the X-chromosome and heterozygous females show a 50% reduced TBG level (215). The deficiency is not the result of a loss of affinity to T_4, but a real TBG deficiency (222). Familial partial TBG deficiency, again linked to the X-chromosome has also been reported (196,224,225). An association between familial TBG deficiency and Graves' disease has been proposed (225).

Familial TBG elevation is found as well (218,223,226). Mostly it is X-linked. Heterozygous females only show a moderate elevation (218). Sometimes no familial relationship can be found (226).

Though several aspects of the metabolism of T_4 and T_3 are changed in cases of TBG deficiency or TBG elevation, the patients are euthyroid and the daily production of T_4 and T_3 is normal (218).

b. *Age dependency of the TBG level*

The TBG level shows a large variation with age. Cord blood levels are above normal adult levels (217). The TBG levels remain elevated until puberty (219), after which there is a drop to normal adult levels. TBG levels are also elevated at advanced age, reaching cord blood values again (219).

c. *Changes by steroids*

Endogenous or exogenous estrogens enhance the level of TBG (217,220,221,223). Especially during pregnancy or oral contraceptive treatment the elevation may be pronounced. The rise of the TBG level is about linear with the duration of the pregnancy (217). Androgens lower the TBG level (223). Dexamethasone, even in a single dose, augments the TBPA level (227). The elevation remains present after a single dose for at least three days.

d. *The influence of the thyroid function*

Most authors mention a small rise of TBG in hypothyroidism (218,219) and a lower TBG in hyperthyroidism (220,221). T_4 medication in normals lowers the levels of TBG and TBPA (228).

e. *The influence of other diseases or medications*

In general, the stress of illness is associated with lower serum binding of thyroid hormones (24,229). TBG elevations are known to occur in a number of diseases, of which acute intermittent porphyria, cirrhosis and hepatitis are well known (218,220). Long-term perphenazine treatment is also known in this respect (218). The most common disease associated with low TBG levels is the nephrotic syndrome (220,230). Here, large amounts of TBG are lost in the urine (220), together with large amounts of T_4.
Several drugs are known to compete with T_4 and/or T_3 for binding sites. Especially anti-inflammatory drugs show this effect. Sulpyrine, acetyl salicylate, salicylate, oxyphenbutazone, indomethacin, phenylbutazone, flufenamic acid and mefanamic acid all significantly displace T_4 from binding proteins, resulting in a higher uptake in vitro of T_4 by muscle (231) or by resin (231). Long-term medication with these drugs in the rat lowers the protein bound iodine (PBI) and increases the fractional turnover rate of T_4 (231).

Veronal, phenylhydantoin, merthiolate and 8-anilino-1-naphthalene-sulfonic acid (ANS) show the same competitive

potency (207,232,233). Naturally, iodothyronine analogues are also active in this way.

All these compounds seem to interfere with the binding sites of TBG and the T_4 binding sites on TBPA (207,233). They probably do not interfere with the T_3 binding sites on TBPA, as proven for salicylate and tetraiodothyroacetic acid (207).

f. *The influence of the nutritional state*

Severe protein-calorie malnutrition leads to a decline in TBPA level (234) and albumin level (235), whereas the TBG content remains stable or becomes slightly elevated (235). Even unbalanced nutrition already results in a TBPA decrease, as judged from retinol binding protein measurements (236).

1.5.3 *The role of the binding proteins*

The serum binding proteins function as a buffer. In the first place the effect of sudden changes in hormone secretion on the free hormone levels will be strongly diluted. In the cascade of stimulations and reactions from TRH to T_4 and T_3 secretion, the relative amplitudes of the successive reactions become progressively larger. So, a minute change in TRH secretion is eventually followed by a large change in the thyroid hormone secretion rate. By means of the binding proteins, the organism is able to damp almost completely the amplitude of the next step: the change in free hormone level.

Secondly, the distribution of the hormones becomes uniform. During its way through the periphery the serum levels of the free hormones will be kept constant, offering each cell the same hormonal impulse.

Because of its high affinity, the TBG level strongly correlates with the total T_4 concentration (219); except at old age (219), where the T_4 level is greatly influenced by changes in the hypothalamic-pituitary control system. The less strongly bound T_3 does not correlate with the TBG level in the normal range (219); large rises in TBG as in pregnancy are followed, however, by rises of T_3 (217).

1.6 THE THYROID HORMONES IN THE TARGET CELLS

1.6.1 *Transcapillary movement*

In order to reach the intracellular compartment the hormones first have to aim for the intercellular space. Lymph from various regions of the body contains protein bound thyroid hormone and albumin in the same relative amounts as in serum, indicating comparable rates of transcapillary movement (237). However, the absolute amounts differ in various regions.

In hepatic lymph the same levels as in serum are found, whereas intestinal lymph and popliteal lymph show ratio's of about 0.75 and 0.40. The levels in intestinal lymph show considerable variation, inversely related to the flow rate (237).

In all lymph samples the free thyroxine level is comparable to the serum value (237), indicating that all cells are supplied with the same amount of free hormone. The hormone reaches the lymph primarily by transcapillary movement of free hormone, as indicated by measurement of the specific activities of T_4 and albumin in serum and lymph after injection of radioactive tracers.

The equilibrium for T_4 and albumin is reached in intestinal lymph after 8 and 26 hours and in popliteal lymph after 32 and 60 hours. The fractional rate of transcapillary movement of T_3 is higher than of T_4 (237), due to its higher free proportion. When the free T_4 level in serum is suddenly raised by i.v. injection of T_4, the transcapillary movement of T_4 is raised likewise (237).

In hepatic lymph, however, the equilibrium for T_4 and albumin is reached much earlier: after two hours for both compounds (237). This indicates that the fractional rate of movement of proteins in this organ into the interstitial fluid is very high and that the hormones reach this space mainly in the protein bound form.

Only the free hormones can enter the cell, probably by a diffusion process. In rat liver the inward rate of diffusion is about 6%/min of the total T_4 present in the liver outside the cells. The backward diffusion rate amounts to 1%/min of the T_4 present inside the cells (238). As about 5 times more T_4 is present inside the cells, the net rate

of transfer is only minimal. This means that the cytoplasmic concentrations of the free hormones are very similar to the extracellular levels.

1.6.2 *Cytoplasmic binding*

In the cytoplasma T_4 and T_3 are bound by specific binding proteins. In the human liver three of these proteins binding T_4 and two binding T_3 are identified (239). The exact role of these proteins is obscure, but they certainly form a buffer against sudden extracellular changes in hormone levels. Besides that, they form an intracellular pool, from which different cell organelles are supplied with the hormones.

The relative amounts of these cytoplasmic binding proteins differ amongst various tissues, resulting in different contributions per tissue to the total distribution spaces of T_4 and T_3.

1.6.3 *Nuclear binding*

Nuclear binding sites, specific for thyroid hormones, were demonstrated in various tissues (52, 240,241). The sites were highly specific for T_3, the affinity for T_4 being 20-fold smaller (242). The T_3 binding sites showed a very limited capacity. In the case of rat liver the capacity was about 4 femtomol T_3/100 μg DNA with an estimated equilibrium association constant of about 3×10^{10} M^{-1} (243).

Kinetic studies in rat liver indicate that most of the nuclear T_3 can freely exchange with cytoplasmic T_3; only minimal amounts, if any, are irreversibly cleared within the nucleus (242). Cyanide, fluoride, dinitrophenol or iodoacetate do not influence nuclear binding of T_3, nor is the presence of cytoplasmic binding proteins a requirement (244,245). The binding material can be eluted from the nucleus with 0.4 M KCl at pH 8.0 and turns out to be a chromatin-associated protein with a molecular weight of 60,000 (244), without histone protein properties (246).

Nuclear binding sites, similar to those present in liver and kidney, were also demonstrated in the rat pituitary (52,246) as well as in all other organs studied (247).

The nuclear binding of thyroid hormones is closely related to the hormonal effect. The thyromimetic properties of thyronine analogues are closely related to their abilities to displace nuclear T_3 (246,248), whereas the binding capacity per nucleus parallels the metabolic dependency of thyroid hormones of different organs (247) or of a tissue at different stages (249). With regard to the feedback phenomenon at the level of the pituitary, the specific nuclear binding of T_3 by these cells is of great importance. Human lymphocytes contain nuclear binding sites similar in their affinity to T_3 as rat pituitary cells (241) and they may provide a good human model. Human polymorphonuclear leukocytes show comparable nuclear binding (250). In this human material, like in rat liver, the presence of cytosol binding proteins is not a prerequisite for nuclear T_3 binding (250).

In the presence of Mg^{2+} a second set of nuclear binding sites can be demonstrated in the rat (243). The capacity of these sites is some 30 fold higher, but their affinity to T_3 is more than 100 times lower (243).

The binding of thyroid hormones within the nucleus is followed by a sequence of reactions, to be reviewed later, that must be considered responsible for the "hormone effect".

1.6.4 *Binding to other cell components*

Thyroid hormones are bound to other cell structures as mitochondria and microsomes. The binding by microsomes is of interest, as it is believed that deiodination of iodothyronines is performed by this cell fraction.

The higher deiodinative clearance of T_3 and the expansion of its distribution space during cold acclimation are related to the enlargement of smooth endoplasmic reticulum (251).

1.6.5 *Deiodination of iodothyronines*

The thyroid hormones are removed from the body in several ways. Some conjugation occurs in the liver, followed by biliary excretion

(251) and fecal loss (166) or by urinary loss (252). Another part of the thyroid hormones is removed unchanged by renal filtration (252,253). The most important route of elimination is through deiodination.

When a iodothyronine molecule is deiodinated, the iodine atoms are removed step by step. Between these steps the molecule may escape from the deiodinating systems. So T_4 can be monodeiodinated to T_3 or reversed-T_3 (rT_3) (see figure 1-5). In this way extrathyroidal production of T_3 from the less potent T_4 substantially adds to the total daily T_3 production.

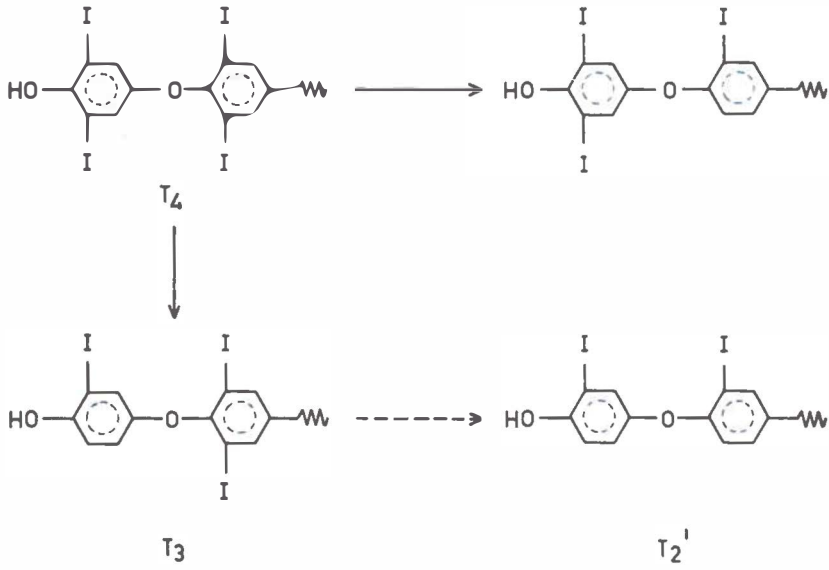


Figure 1-5. The peripheral deiodination of the thyroid hormones. The horizontal reactions are catalyzed by the "inner" enzyme, the vertical reactions by the "outer" enzyme.

The deiodinative production of T_3 is confined to the microsomal fraction of the cell (254). Evidence has been obtained that at least two enzyme systems will be present in the human that monodeiodinate T_4: one producing T_3 and the other rT_3.

Data on these enzymes in rat liver are available now. The broad pH optium of T_3 generation is from 6 to 8 (255,257). The generation of T'_2 from T_4 parallels the pH curve of the T_3 generation and about equimolar amounts of T_3 and T'_2 are formed (255). This is in accordance with the similar production rates of T_3 and rT_3, measured in T_4 substituted hypothyroid patients (256). Generated rT_3 is rapidly metabolized to T'_2 (255), except at pH values of about 5 and 9 (255,257). This means that the pH range of rT_3 generation is slightly broader than that for T_3 and T'_2 generation. The T_3 generation is strongly inhibited by rT_3. This inhibition results from the high affinity of the enzyme for rT_3 ($Ki = 3.10^{-8}M$) compared to that for T_4 ($Km = 2.10^{-6}M$) (255). T_3 is hardly metabolized to T'_2 (255) and does not inhibit rT_3 generation (257).

These data can be explained in terms of two different enzymes, the first one removing iodine from the outer ring ("outer enzyme") and the other from the inner ring ("inner enzyme"). The outer enzyme preferably removes iodine not from T_4 but from rT_3, whereas the inner enzyme almost exclusively removes iodine from T_4, leaving T_3 untouched (see figure 1-5). This means, that the intracellular rT_3 level will remain low enough to allow T_3 generation, as long as all the rT_3 which is generated from T_4 can be metabolized at once to T'_2. When the activity of the outer enzyme falls below the activity of inner enzyme, T_3 generation will sharply decline.

1.7 THE INFLUENCE ON THE DEIODINATING ENZYME SYSTEMS

1.7.1 *Age effects*

In the newborn the enzyme responsible for T_3 generation from T_4 is not or only suboptimally active. This is known for other enzyme systems. It results in low T_3 levels (99,258-261). As a consequence the TSH levels are high, in order to stimulate thyroidal T_3 production for compensation. This, in turn, results in increased T_4 levels (97,258-261), that are responsible for the increased levels of rT_3, as the rT_3 generating deiodinase is already present (258,260-262).

Shortly after delivery, the TSH surge is followed by a rapid increase in thyroid hormone levels. T_3 and T_4 reach peak levels after one day (258,259). By that time the T_3 level is sligthly higher than of normal adults, but the T_4 level is at least twice the normal adult level (258,259). As hardly any rT_3 is produced by the thyroid, the level of this compound does not change much (258). The T_3 surge is followed by a decline, paralleling the TSH level, to a normal adult level after 3 or 4 days (99,258). After about a week T_4 and rT_3 have slowly declined to the upper normal adult values, whereas T_3 has slowly increased to its upper normal adult level. Whether the T_3 generation is optimal by that time is hard to say. After six weeks the free T_3 level is higher than in adults or pregnant women at term (99), whereas the free T_4 level is the same as in normal adults. The total T_3 level remains somewhat elevated, parallel to the TBG level, until puberty (263).

In ages over 60 the setpoint of the pituitary thermostat becomes progressively lower. The absolute thyroidal iodide uptake decreases, but the free T_4 level remains in the normal range (263,264). The T_3 level, however, decreases (60,263-266). At the same time the rT_3 level rises (267). Probably the equilibrium between the T_3 and rT_3 generating systems becomes unbalanced. Whether this phenomenon must be partly explained by malnutrition (see below) is unknown. The lower T_3/T_4 ratio at old age is thus due to a lower setpoint as well as to a decreased T_4-T_3 conversion.

1.7.2 *Effects of thyroid hormones*

The amount of T_3 generating enzyme in rat liver is strongly thyroid hormone dependent. In thyroidectomized animals the enzyme concentration closely follows the serum T_3 level, both for T_4 substitution and T_3 substitution (268). Doses of T_4 or T_3 resulting in clear hyperthyroidism, enhance the T_3 generation two- to threefold (257,268). In this way hyperthyroidism substantially enhances peripheral T_3 generation, thus aggravating the hypermetabolic state.

1.7.3 *The influence of pharmacological substances*

In the T_4 substituted thyroidectomized rat, thiouracil compounds inhibit the conversion of T_4 to T_3 resulting in a rise of the serum T_4 and TSH levels, a drop of the T_3 level (269) and a decrease of the fractional removal rate of T_4 (270). Together with this, the biliary excretion of both T_4 and T_3 about redoubles (271), resulting in an increased fecal fractional removal of T_4 and T_3 (270).

In T_4 substituted man propylthiouracil inhibits peripheral T_3 generation as well, resulting in lower levels of T_3 (272,273) and augmenting the basal TSH levels and/or the TRH stimulated TSH response (272,273). The T_4 levels do not change significantly. Thiouracil compounds depress peripheral T_3 generation mainly through inhibition of enzyme activity (274) and not by depression of enzyme content. Methimazole does not change peripheral T_3 generation (273).

Another drug, diphenylhydantoin, increases liver T_3 generation in the rat by enzyme induction (275).

Phenobarbital does not change the content of T_3 generating enzyme (275), but the serum T_3 level decreases, in contrast to the T_4 level (275).

Dexamethasone decreases serum T_4 and T_3 in the rat (275), primarily through hypothalamic action. At the same time the T_3 generating enzyme content of the liver sharply decreases (275). Dexamethasone does not inhibit the enzyme activity (275).

In man dexamethasone treatment leads to lower levels of serum T_4 and T_3 (82), whereas the rT_3 level rises (276). Even a single dose is followed by these changes of T_3 and rT_3 in T_4 substituted normals (206,277). In Graves' disease dexamethasone has the same effect (276).

Compounds containing an iodine-substituted benzene ring, such as in use as radiographic contrast media, may compete with T_4 at the T_3 generating enzyme. In man Na-iopanoate, in use for cholecystography, lowers the serum T_3 level and augments the serum rT_3 shortly after use. Parallel with these changes the basal level of TSH rises (227). The serum level of the contrast medium stays high

enough for at least one week to inhibit peripheral T_3 generation substantially. After two weeks the levels of the thyronines are back to normal again.

Other contrast media, bearing iodine substituted benzene rings, such as diatrizoic acid (urography) and ioglycamic acid (cholangiography) do not inhibit peripheral T_3 generation (227). These compounds bear a benzoic acid substitution and might therefore show negligible affinity to the T_3 generating enzyme.

1.7.4 *Influence of diseases*

a. *Influence of liver disease*

As the liver plays a major role in the T_3 generation from T_4, liver disease results in a reduction of the daily T_3 production.

In a group of patients with alcoholic hepatic cirrhosis the serum T_4 level was normal, but the serum binding was decreased, yielding a higher free T_4 level (278). At the same time the serum T_3 level was reduced and the TSH level increased. The T_3 level correlated positively with the albumin level and negatively with the serum bilirubin level, both indicators of the degree of the disease (278). The rates of production of T_4 and T_3 were decreased, but the decrease in T_3 production was much more pronounced than that of T_4 (278).

The same pattern was found by other investigators. In addition high rT_3 levels were found, but as a result of a decreased metabolic clearance rate the daily rT_3 production remained comparable to that in normals (150).

When liver diseased patients were grouped according to the severity of their disease, the whole pattern of changes could be followed (279). In the least affected group the levels of T_4 and T_3 were reduced, but still in the normal range, whereas the rT_3 level was at the upper end of the normal range. The TBG level was elevated. In the next stage the T_4 level was reduced a little more, but the T_3 level was

very low. The rT_3 level was greatly increased to three times the normal level. The TBG level had returned to normal. In the last group both T_4 and T_3 were very low, TBG remained normal, but the rT_3 level had returned to within normal limits. In the last two groups additional effects, due to severe illness, are active, as reviewed below.

b. *Influence of other diseases*

Severe chronic non-thyroidal illness is associated with decreased peripheral T_3 generation (25,260,280). The T_3 levels are low (24,25,260,280,281) and the rT_3 levels are high (25,260,267). During illness the TSH response to TRH is within the normal limits (24), indicating a downward shift in the setpoint.

Acute illness results in the same inverse shift of serum T_3 and rT_3 concentrations (282,283), though after myocardial infarction the rT_3 levels generally do not exceed normal levels (282,284). With convalescence all levels return to normal (283,284). The same changes are observed after experimental falciparum malaria (285) and infective febrile illnesses (229). During the acute phase, the TRH test remains normal, regarding the TSH response (229,285) as well as the T_3 response (229).

c. *Malnutrition effects*

The same pattern of hypothalamic and peripheral changes as in severe illness, was found in anorexia nervosa (267). Complete fasting of volunteers resulted in similar changes (286-288), that rapidly reversed upon refeeding (286,288).

A carbohydrate-free hypocaloric diet resulted in T_3 levels comparable to complete fasting, but rT_3 levels did not change. Isocaloric replacement in this hypocaloric diet of 50 gr of carbohydrate prevented not only the changes in rT_3 but also those in T_3 (287). The serum T_3 level in the carbohydrate-free experiment correlated positively with the glucose level and negatively with the blood ketones level (287).

In severe protein-calorie malnutrition low levels of T_3 are found likewise (235). Even moderate deviation of a normal nutritional status is accompanied by slightly lower T_3 levels, but much of this decrease is due to a TBPA fall (236).

d. *Influence of surgery*

Stress resulting from general anaesthesia is followed by a rise of the rT_3 level paralleling a fall of the T_3 level (24,289,290). Surgical manipulation of the thyroid, as with parathyroidectomy, obscures these effects in some degree. In this situation the drop of the T_3 level is balanced by enhanced thyroidal secretion, indicated by the rise of the T_4 level (289).

e. *Glucocorticoid hypothesis*

Taken together, the inhibition of the peripheral T_3 generation is strongly related to situations in which the glucocorticoid levels are high: dexamethasone medication, stress, fasting, malnutrition, dietary carbohydrate shortage.

The observation that in all instances, in spite of low T_3 levels, the TRH test remains normal, fits the "glucocorticoid theory", as glucocorticoids are known to lower the "setpoint" by blocking TRH release.

Arguing against this explanation are the observations made in two groups of patients undergoing abdominal hysterectomy (290). One group received general anaesthesia, the other epidural analgesia. In the latter, in contrast to the former, serum cortisol did not rise. But the T_3 level fell in both groups. The T_3 levels were not different, except at six hours from the start of the operation, when the T_3 level in the general anaesthesia group was lower and thus more decreased than in the analgesia group.

The authors conclude that cortisol is not involved in the decrease of peripheral T_3 generation. But, though in the analgesia group the cortisol level did not rise much, it certainly did not fall as would have been normal because of the daily rhythm. So the difference in cortisol

response was only a matter of quantity. That initially the fall of the serum T_3 levels did not differ, may have been caused by a shift of the thyroid hormones to the serum compartment during general anaesthesia. This shift provoked a rise in serum T_4 (290) and could have obscured a difference in the fall of serum T_3. Indeed, by the time that the serum T_4 had normalized, the serum T_3 levels became different (290).

Whether the glucocorticoids are really pivotal in this fast acting system is of minor importance. Relevant is, that in those situations where energy is scarce or is needed elsewhere, all thyroid hormone activated, energy consuming, processes can be cut off at once. This is done in a way that is as magnificent as it is simple. The complete machinery of hormone secretion is short-circuited by arresting the TRH release. At the same time almost all the hormonal potency that is already present, is wasted through rT_3 generation and enhanced fecal loss.

1.7.5 *Adaptation to cold*

In the rat, living in a cold environment is accompanied by microsomal enlargement and an increased microsomal binding of T_3 (251). This might indicate an enhancement of peripheral T_3 generation, to meet the demand resulting from the enhanced T_3 metabolism. Its fits the observation of raised serum T_3 levels and normal TSH levels in inhabitants of a Japanese district, who use no adequate heating in winter (96).

The exposure of man to extreme cold for some days is too abrupt to provoke a sufficient change in microsomal T_3 production and the enhanced need of thyroid hormone must be substituted with an enhanced thyroidal hormone production through TSH elevation (18).

1.7.6 *Congenital deficiency of T_3 generation*

In theory a defect of the peripheral T_3 generating enzyme is feasible. Some of the cases of thyroid hormone resistance might even

be compatible with this kind of defect. As early as in 1957 such an enzyme was postulated in a case of sporadic goiter that poorly responded to desiccated thyroid but did well on T_3 medication (291). Data, to support this view, obtainable nowadays, are of course lacking.

1.7.7 *Iodide production from thyroid hormones*

Deiodination of thyroid hormones is the quantitatively most important route of degradation. Most of the thyroxine is eventually degradated to thyronine and thyroacetic acid and cleared by the kidney (292). Factors influencing the generation of T_3 and rT_3 also influence the overall deiodinative degradation.

In hyperthyroidism the metabolic clearance rates of T_4 and T_3 are increased, in hypothyroidism they are decreased. In human lymphocytes the degradative iodide liberation can be followed. In hyperthyroidism the fractional cellular uptake of labelled T_4 and T_3 is increased, as well as the fractional iodide production. In hypothyroidism the fractional uptake is also increased, but the fractional iodide production remains normal (293). Upon treatment the uptake and deiodination normalize (293).

1.8 THE HORMONE EFFECT

Nuclear binding of T_3 is followed by a sequence of effects (294,295). The available data have been reviewed recently (296). The first effect is the increase of nuclear RNA production, soon followed by an increase of a number of enzymes, of which RNA polymerase is the first to react (295). Along with the overall protein synthesis the mitochondrial oxygen consumption rises (294,295).

In experimental animals some enzymes are excellent markers of the thyroid hormone effect. Glucose-6-phosphate dehydrogenase, alpha-glycerophosphate dehydrogenase, malic enzyme and NADPH-cytochrome-C reductase are well known in this respect. In thyroidectomized animals the amounts of these enzymes increase as long as the nuclear binding sites for T_3 are occupied after T_3 injection

(295). From the moment that the nuclear sites are empty again, all the parameters of thyroid hormone action decrease with a similar exponential rate (294,295).

This fractional decay rate with a half-time of about 4.5 days in hypothyroid rats and about 3 days in euthyroid rats (294,297), is much smaller than the rate of removal of T_3 and the rates of destruction of the enzymes. This indicates that there must be a long-lived messenger of thyroid hormone action (294). In the interpretation of kinetics of thyroid hormone effects, this concept is very important.

Different tissues show large differences in the T_3 exchange rates with plasma (295). Thus, the kinetics of the hormone effects will be different for each tissue after a sudden change in serum thyroid hormone level.

The relative potencies of different thyroid hormone analogues can be studied by liver enzyme induction in experimental animals. Using a cell culture system of rat pituitary tumor cells, the peripheral influences can be omitted largely and intrinsic potencies can be measured (298).

In this system free T_4 has only 8% of the intrinsic activity of free T_3, whereas T'_2 and rT_3 have negligible activity (298). These cultured cells respond to physiologic concentrations of T_3 by an enhanced de novo growth hormone production (299). The growth hormone production starts to accelerate about one hour after nuclear binding of T_3 and becomes maximal after 8.5 hours. Studies with actinomycin-D, 3^1 - deoxyadenosine and cycloheximide indicate that the production and accumulation of an RNA species is the rate limiting factor (299). This RNA could be shown to be growth hormone mRNA (300).

In the human several parameters of the hormone effect are obtainable. The most direct approach is the examination of the TSH release inhibiting effect by means of a TRH-test. Non-invasive measurements include oxygen consumption (BMR), pulse rate, arterial sound timing, achilles tendon reflex relaxation timing and urinary excretion of substances as hydroxyproline. Measurements in

serum of proteins such as sex hormone binding globulin (301) or red blood cell enzymes (302,303) provide good parameters as well. Most of these parameters are influenced not only by thyroid hormones. Accordingly their specificity is low; yet they have value when studying groups of subjects or changes in the same subject.

Measurement of the cardiac pre-ejection period (304,305), or even simpler the QKd interval (306-308), gives information that closely follows the best parameter of thyroid hormone effect: the TRH-test. The QKd interval may turn out to be specific enough to get some popularity, especially as it can be readily measured.

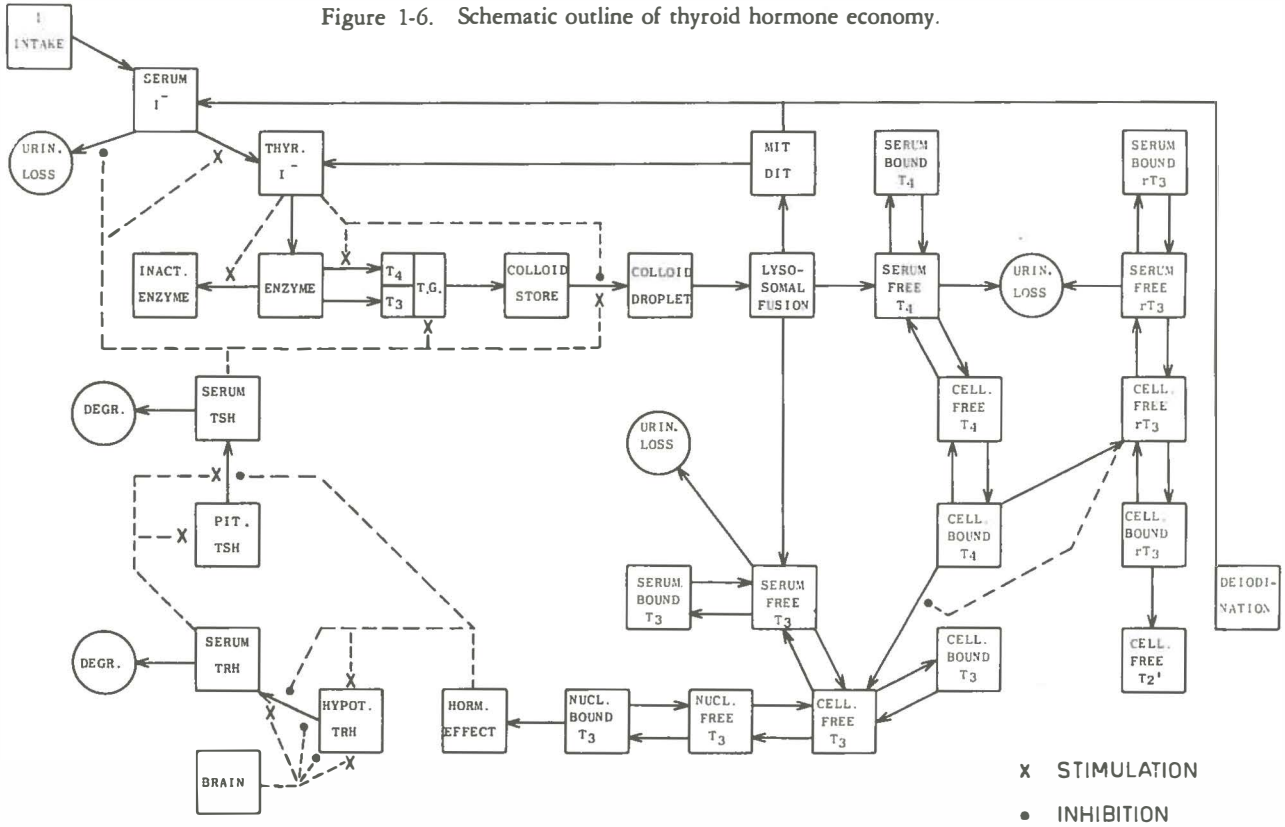
In those cases where the TRH test seems in conflict with the serum thyroid hormone levels, the measurement of hormone effect parameters may provide important evidence. In this way it can be shown, that hypothyroidism is present in anorexia nervosa despite normal TSH levels (124), that hyperthyroidism is present in selective pituitary thyroid hormone resistance (47) and that there is euthyroidism in partial peripheral thyroid hormone resistance (309,310). Even the gradual decline of the hormone effect during starvation can be monitored by the decrease in heart rate and the increase in chillinessscore (288). The hormone effect returned to normal two days after refeeding was started (288).

1.9 SCHEMATICAL OUTLINE

The pathways of the regulation of thyroid hormone action are schematically depicted in figure 1-6. It is easily noticed that the cascade of stimulations with growing amplitudes, inhibited by the thyroid hormone effect, is formed by the left side of the figure. Once the thyroid hormone has been secreted, the amplitudes are progressively buffered, as shown in the right side of the figure, until eventually the hormone effect is exerted.

For reason of simplicity, the intrinsic activity of T_4 is omitted in the scheme. The changes brought about by inborn or acquired abnormalities, as well as by physiologic and iatrogenic influences, can be followed through this "regulatory cycle" easily. Though not visualized, the hormone effect enhances the clearance of TRH, TSH and the iodothyronines.

Figure 1-6. Schematic outline of thyroid hormone economy.



To indicate the relative magnitudes of the various components of the regulatory cycle in normal adults, data from a few studies are provided in table 1-1. Some comments should be made regarding the data of this table. Most authors (311-314) calculate the T_4 production and the T_4 space higher: about 85 μg/d and 11 liters. Further, some new reports from Germany and England, based on the use of high-titer antisera, indicate that the normal level of rT_3 may be half the value indicated in the table (261,262,279,315). This means that the other parameters will be half as high also.

TABLE 1-1. Some parameters of thyroid hormone regulation in normals.

	TRH	TSH	T_4	T_3	rT_3
Daily production		104.3 mU/d	64.2 µg/d	30 µg/d	37 µg/d
Thyroidal production			100 %	30 %	0.9 µg/d
Peripheral production			0 %	70 %	
Distribution volume	15.7 l		8.7 l	39.3 l	
Total serum level	15 pg/ml	1.5 µU/ml	7.6 µg/dl	137 ng/dl	48 ng/dl
Free serum level			1.3 ng/dl	240 pg/dl	
Metabolic clearance rate		50.7 ml/min	0.84 l/d	22.9 l/d	76.7 l/d
Fractional removal rate	13 %/min		9.7 %/d	60.3 %/d	
Extra-thyroidal pool			665 µg	54 µg	
Adopted from	*12,21*	*56*	*228*	*228,317*	*150*

As stated before, the distribution of T_4 and T_3 over the different organs differs markedly. Except for the extracellular fluid, T_4 is almost restricted to the liver i.e. 35% of total T_4, whereas only 15% of the total T_3 is in the liver (294). As the liver, in contrast to other compartments, exchanges thyroid hormones rapidly with serum, the kinetics of T_4 and T_3 will differ greatly. Besides that, the cellular space of T_4 is rather stable, in contrast to the space of T_3, which markedly increases in hyperthyroidism or T_4 oversubstitution (228,312). So, in hyperthyroidism and T_4 oversubstitution, the enhanced metabolic clearance rate of T_4 is paralleled by its fractional removal rate (228,312). But the T_3 kinetics in slight hyperthyroidism, resulting from T_4 oversubstitution with 300 μg/d, are characterized by an enhanced metabolic clearance resulting from space enlargement and

unchanged fractional clearance (228). Only with higher T_3 levels, as in overt hyperthyroidism, the fractional removal rate inclines as well (312).

Farmaceuticals as phenobarbital enhance the metabolic clearance rate of T_4 and T_3 through acceleration of the fractional removal, leaving the distribution spaces unchanged (316). Phenobarbital induces microsomal enzymes, leading to enhanced microsomal binding and acceleration of the clearance, especially via the fecal route (316). Liver damage as in mild hepatic cirrhosis, does not change the metabolic clearance rates of T_4 and T_3, though their production rates decrease (150). In this situation the rT_3 production may remain unaltered, but the metabolic clearance rate is greatly reduced already (150).

Chapter 2

A SIMPLE KINETIC MODEL

2.1 THEORETICAL CONSIDERATIONS

Thyroid hormone has a significant effect on several kinetic parameters normally used in models to describe changes in levels or activities. For instance, the rate of clearance of thyroid hormone will not be constant, but a function of the actual thyroid hormone effect. This means that a thyroid hormone model would become rather complex, when it has to account for all these interrelationships. However, when one wants to study the changes in the control system brought about by disease or medical manipulations, a simplified model can be used as long as no exact quantitative changes are aimed at. Such a simple model, equipped with constant parameters, will be described.

Some fundamental principles on which the model is built have been indicated in chapter 1. Just as for rT_3, no intrinsic activity will be assigned to T_4. In the absence of T_3 there may be some intrinsic activity of T_4 observable, but the affinity for T_3 of the nuclear binding sites is so much higher that in the presence of T_3 hardly any significant extra effect of T_4 has to be expected.

Moreover we have seen that the levels of free T_3 and free T_4 within the cells are equal or at least very similar to the serum free hormone levels. Therefore the serum free hormone levels are substituted for the intracellular free hormone levels. This means that in case of normal serum binding proteins we can substitute the average total serum levels for the free hormone levels, or even the total amount of hormone in the compartment to which the serum belongs.

Marked changes in the peripheral T_3 production rate are found only in acute stress-situations or severe chronical disease. As we want to use a constant fractional rate of conversion of T_4 to T_3, we thereby accept that no predictions can be made in these conditions without a modification of the model.

We have compared the pituitary with a thermostat. More technically, the characteristics of a thermostated system have been described in terms of a proportional control loop (318). The pituitary functions in this loop as the comparator, comparing the command signal (TRH) with the signal (thyroid hormone effect). The difference between both signals, called the error signal, is amplified by the comparator producing a control signal (TSH). The control signal drives the servo-generator (the thyroid). The servo-generator again amplifies the control signal, producing a changed signal (hormone secretion) in order to minimize the error signal.

The product of the amplification factors of the comparator and the servo-generator defines the "loop gain" of the system. The term "loop gain" is used in electronics for the total amplification of a signal by the circuit. A control system with a low loop gain tolerates large error signals, but a high loop gain circuit will try to correct even the smallest error.

It is easily recognized that the failure of such a control system can be due to different causes. Firstly, the command signal can be wrong, leading to hypothalamic hypothyroidism or, theoretically, to hypothalamic hyperthyroidism. Secondly, the comparator amplification may become too low to maintain a sufficient loop gain and secundary hypothyroidism develops. Thirdly, the amplification of the servo-generator may decrease, leading to primary hypothyroidism.

One should keep in mind that autonomous TSH overproduction is not synonymous with an increased comparator amplification, as the TSH production is not proportional to the error signal. Also, Graves' disease can not be regarded to result from an increased servogenerator amplification.

2.1.1 *The pituitary*

Let us call the TRH command signal N and the thyroid hormone effect on the pituitary E. The normal control system will seek to match N and E. Therefore N and E will be similar in control terms in normals. Measurement of E in normals gives us this "setpoint", the

reference point on the hormone effect scale. E can be chosen to be measured in real effect parameters as e.g. BMR or QKd (see chapter 1), but this will be equivalent to the free serum hormone level in equilibrium situations, assuming that the sensitivity for the hormone is constant.

The pituitary secretes TSH proportional to the error signal (N-E). If we call the amplification factor A, the serum TSH level S and the fractional removal rate of TSH k_1, we find the TSH level to be

$$S = A(N-E)/k_1.$$

The magnitude of A will depend on the number of thyrotropic cells and their TSH content. Therefore an equivalent for A will be the maximum output of the pituitary, when no inhibition of thyroid hormone is present. By way of a TRH-test we can be informed on the magnitude of the maximum output (M) of the pituitary. After administration of a high dose of TRH, all TRH receptor sites will be occupied for a certain period. The change of the TSH level will be, when AE does not exceed M,

$$dS/dt = (M-AE) - k_1S$$

and therefore the TSH level will be

$$S = [(AN-M)\exp(-k_1t) + (M-AE)] / k_1$$

After a short interval the TSH rise would be

$$\Delta S = (M - AN)(1 - \exp(-k_1t)) / k_1$$

This means that the TSH response is determined solely by the number and content of the thyrotropic cells, as long as the endogenous N exceeds E.

Accordingly, the exaggerated response found in gradually developed primary hypothyroidism must result from hyperplasia. Hyperplasia, an increase of A, is therefore the answer of the control system to a failing thyroid, in an attempt to keep the loop gain as high as possible.

When the maximum TRH stimulation is maintained by TRH infusion, the TSH level will reach a plateau value

$$S = (M - AE) / k_1$$

This level remains constant until the hormone effect changes through the increased thyroid hormone release.

2.1.2 *The thyroid*

The thyroid hormone levels can be described in a similar way. Let us assume the fractional removal rates of the total body pools of T_4 and T_3 to be $(k_2 + k_3)$ and k_4 respectively. A certain part (k_3) of the removal rate of T_4 is based on the conversion of T_4 to T_3, the other part (k_2) concerns all other routes of T_4 removal. When we further call the amplification factor of the thyroid B and assume a constant secretion during the day, the total body pool of T_4 (TT_4) becomes:

$$TT_4 = BS / (k_2 + k_3)$$

If we call the weight ratio of the secretions of T_3 and T_4 b, the total body pool of T_3 (TT_3) becomes:

$$TT_3 = BS\,(b + k_3/(k_2 + k_3)) / k_4$$

These values apply to the equilibrium situation.

When suddenly the TSH level is raised to a constant level of S_1, the thyroid reacts by enhancing its secretion. The change of the T_4 pool becomes:

$$dTT_4/dt = BS_1 - (k_2 + k_3)TT_4$$

which means that the T_4 pool becomes:

$$TT_4 = [B(S_1-S)(1 - \exp(-k_2t-k_3t)) + BS] /(k_2 + k_3)$$

and the T_4 response:

$$\Delta TT_4 = B(S_1-S)(1 - \exp(-k_2t-k_3t)) / (k_2 + k_3)$$

The T_4 level in serum closely correlates with the total body pool of T_4. This indicates that, when the distribution of T_4 over this volume is fast enough, the rise of the T_4 level is proportional to the rise in TSH level and the amplification factor B.

Similarly the T_3 response can be followed. However, because of marked differences in exchange rates of different organs, the distribution volume must be divided in two compartments. The first one (V_3f) includes the serum volume and is in fast equilibrium with the serum. The other compartment (V_3s) only equilibrates slowly. The total production of T_3 enters the fast pool. If we give the fractional degradation rates in both pools the same value k_4 and the

fractional fluxes between both pools k_5 and k_6, we find at equilibrium for the fast T_3 pool:

$$TT_3f = BS\ (b + k_3/(k_2 + k_3))(k_4 + k_6)\ /\ (k_4 + k_5 + k_6)\ k_4$$

whereas

$$k_5TT_3f = (k_4 + k_6)TT_3s$$

If one now neglects the change in the small flux from the slow pool to the fast pool, the response of the fast pool to a suddenly raised TSH level can be calculated to be:

$$\Delta\ TT_3f = B(S_1\text{-}S)(b + k_3/(k_2 + k_3))(1 - \exp(-k_4t\text{-}k_1t))/(k_4 + k_5) +$$

$$k_3B(S_1\text{-}S)(\exp(-k_2t\text{-}k_1t) - \exp(-k_4t\text{-}k_1t))\ /\ (k_2 + k_3)(k_2 + k_3 - k_4 - k_5)$$

When the interval is taken small enough, the increases of the T_4 and T_3 levels are proportional to the initial rates of change: $B(S_1\text{-}S)$ and $bB(S_1\text{-}S)$ respectively. Therefore the ratio of the pool responses is constant initially: $\Delta\ TT_3f/\ \Delta\ TT_4 = b$. This means that the value of b can be calculated from the serum hormone responses when the volumes of the T_4 pool and the fast T_3 pool are known.

2.1.3 *The hormone effect*

The hormone effect is initiated by the binding of T_3 to nuclear receptors. Some compound (E) is formed in relation to the occupancy of the available binding sites. This compound has a long half life and is responsible for the various hormonal effects. In the pituitary one of these effects is the production of the blocking protein, the inhibitor of TSH secretion. The half life of the blocking protein is probably shorter than that of the messenger compound E. Therefore the kinetics of the blocking protein will follow the kinetics of E.

The occupancy (O) of the nuclear sites depends on the serum T_3 level: $O = xT_3$. With rising T_3 levels x will decrease as the occupancy shows a hyperbolic relation to the T_3 level: $O = T_3/(T_3 + P)$. P is a constant, close to the normal T_3 level (297). The production of E is not proportional to the occupancy, but is progressively amplified with a rising O (297). When we call this amplification factor y, the production of E becomes xyT_3. With changes in the T_3 level both x and y will change, but in opposite direction. Therefore their product

xy can be considered to be constant. This constant (C) has the meaning of target organ sensitivity to T_3.

Accordingly, when we neglect the small intrinsic hormonal effect of T_4, the production of E becomes proportional to the fast T_3 pool. At equilibrium we calculate, when k_7 is the fractional removal rate of E:

$$E = C\ TT_3f\ /\ k_7 \text{ or}$$

$$E = ABC(N\text{-}E)(b + k_3/(k_2 + k_3))(k_4 + k_6)/(k_4 + k_5 + k_6)k_1k_4k_7$$

During a constant elevation S_1 of the TSH level, E will increase as a consequence of the T_3 rise. The kinetics of the change are governed by $dE/dt = C\ TT_3f - k_7E$. Finally E reaches its new equilibrium value that is S_1/S_0 times its original value.

2.2 THE MAGNITUDE OF THE PARAMETERS

Some of the parameters needed in the model have been calculated in normal man by others. For the proposed model we take the following approximated values, for a 60-70 kg person:

k_1 = fractional removal rate of TSH = 0.8/h (319)
k_2+k_3 = fractional removal rate of T_4 = 0.12/d (228,313)
k_3 = fractional production rate of T_3 from T_4 in g/g=0.03/d (317)
k_4 = fractional removal rate of total body T_3=0.6/d (228,313)
k_5 = fractional flux from the fast T_3 pool to the slow pool
k_6 = fractional flux from the slow T_3 pool to the fast pool
k_7 = fractional removal rate of the "T_3 effect"=0.2/d (320)
$(k_2+k_3)TT_4$ = daily thyroidal T_4 production = 86 μg (311-313)
b = weight ratio of thyroidal T_3 and T_4 production =0.1 (149)
V_4 = distribution volume of T_4 = 11 L (311-313)
V_3 = total distribution volume of T_3=$V_3f + V_3s$ = 40 L (228,311,312).

Some other parameters can be deduced directly from the given values. The daily thyroidal T_3 production will be 8.6 μg, the total extrathyroidal T_4 pool 717 μg and the total T_3 pool 50.2 μg. These values are comparable to the actually reported average values (228,311).

We now have to find the values of k_5, k_6 and the volumes of the fast and slow T_3 pools.

We have seen that the initial rises of the fast T_3 pool and the T_4 pool will show a fixed ratio b. Accordingly, the ratio of the rises of the serum level would be bV_4/V_3f. This ratio has been reported to be 1/20 (201,202), which means that $V_3f = 22$ L and therefore $V_3s = 18$ L. At equilibrium, the fast T_3 pool is therefore about 27 μg.

If we accept that the fractional degradation in the slow and fast pools of T_3 are equal (k_4) and that a fair approximation of k_6 is 0.1/d, then k_5 must be 0.6/d.

2.3 APPLICATION OF THE MODEL

When using the proposed simple model, one should keep in mind that several of the parameters are not constant in reality and that the model is not equipped with lag phases. These lag phases can differ for various systems. For instance, the lag phase in man for the initiation of the feed back inhibition of T_3 on the pituitary seems to be about one hour (321), which is much smaller than the lag phase of about 10 hours of some liver enzymes in the rat (297). As a consequence, the model is unable to predict all changes over a short initial period correctly. Yet the model should be usable for equilibrium situations, as well as for changes over intervals of several hours or days.

2.3.1 *Application on substitution doses*

a. *Thyroxine substitution*

As the fractional removal of T_4 is very low, one can consider the absorption of one oral dose (D) per day during long-term substitution constant during the whole day. Therefore $dTT_4/dt = D - 0.12\ TT_4 = 0$. This means that $TT_4 = D / 0.12$.

When no functional thyroid tissue is present, the total T_3 production stems from T_4 conversion. This production will be constant during the day and accordingly the fast and slow pools of T_3 remain in equilibrium in such a way that $k_5TT_3f = (k_4+k_6)TT_3s$. This means

that the production will match the degradated amount of the total T_3 pool: $0.6TT_3 = 0.03\ D/0.12$
Therefore the total T_3 pool will be $0.25\ D/0.6$.
In normals this amount is 50 μg, which means that the daily absorbed dose D must be 120 μg.

The absorption percentage of such an oral dose of T_4 has been reported to be 75% (322). Consequently, the average daily needed oral dose must contain 160 μg. This value is very close to the observed suppressive substitution dose, varying from 100 to 200 μg, with an average of 169 μg (323) and 150 μg (324).

The serum T_4 level will be $120/86 = 140\%$ of the normal level in the correctly substituted patient. This elevation is well known for T_4 substitution (323).

b. *Triiodothyronine substitution*

Because T_3 is metabolized so rapidly, one can not consider the absorption of T_3 to be constant during the day, even if divided doses are given. Judged from the kinetics of the serum T_3 level, the absorption of an oral dose takes about 4 hours to be completed (325-327). When it is assumed that the absorption rate is constant during these 4 hours and that the small flux from the slow pool to the fast one is constant during the whole day, we find the change of the fast pool during the absorption period:

$$dTT_3f/dt = -1.2TT_3f + 0.1TT_3s + 6D$$

and afterwards:

$$dTT_3f/dt = -1.2TT_3f + 0.1TT_3s$$

With one dose per 12 hours, the fast T_3 pool will be:

$$TT_3f = -3.66\ \exp(-1.2t) + (6D + 0.1TT_3s)/1.2$$

during absorption and

$$TT_3f = 2.01\ \exp(-1.2t) + 0.1TT_3s/1.2$$ thereafter.

We considered the flux from the slow pool to be constant, which means that this amount can be calculated as if the dose had been infused during 12 hours. We find a value

$$TT_3s = 0.2D/1.3$$

As a consequence of the varying T_3 levels, E will not be constant. We can calculate E to be:

$$E = C[-20.33D \exp(-0.2t)+3.66\ D \exp(-1.2t)+(6D+0.2D/1.3)/0.24]$$

during absorption and afterwards:

$$E = C[10.35D \exp(-0.2t)-2.01D \exp(-1.2t)+0.2D/0.312]$$

The minimal value of E will be reached shortly after the start of an absorption period. At that moment $dE/dt = 0$. We calculate the interval to be 0.73 hours or about 45 minutes; the minimal value of E is 8.96 DC. In the same way we can calculate the maximal value of E, which turns out to be 8.99 DC. This indicates that the hormone effect hardly changes during the day, although the serum T_3 level fluctuates considerably.

We know that in normals $TT_3f = 27\ \mu g$ and therefore $E = 135$ C. Accordingly the absorbed dose must be 15.1 μg. With an absorption percentage of 95% (313), the oral substitution dose should be about 16 μg twice a day. This is close to the oral dose of 29 μg once a day that was needed in normals to decrease the TSH response in a TRH-test to half of the original value (324). Complete suppression is found with doses between 40 and 50 μg (324, 328). This observation indicates that the T_3 disposal rate increases with rising doses, probably through enhanced urinary loss during the periods in which the T_3 levels are high. Therefore the T_3 level found just before the next dose does hardly change with single daily doses up to about 75 μg (324,328).

2.3.2 *Resumption of TSH secretion after stopping medication*

a. *Triiodothyronine medication stop*

In order to perform total body scintigraphy in patients treated for thyroid carcinoma, the thyroid hormone medication must be stopped to ensure an elevated TSH level during the study. From our department data have been reported on the resumption of TSH secretion after stopping a regimen of 50 μg T_3 twice a day (320). On the average the TSH secretion started 6.7 days after the last dose.

We have seen that the fast T_3 pool during the period of absorption of a dose is:

$$TT_3f = -3.66\ D\ \exp(-1.2t) + (6\ D + 0.2\ D/\ 1.3)/1.2$$

Therefore at the end of this period $TT_3f = 2.13\ D$. It can be calculated in an analogous way that the slow T_3 pool during absorption is:

$$TT_3s = -7.00D\ \exp(-0.6t) + 3.66D\ \exp(-1.2t) + 4.87D,$$

which means that $TT_3s = 1.54\ D$ at the end of the absorption period. If we now neglect the small flow from the slow pool to the fast pool, until k_6TT_3s becomes k_5TT_3f, we find:

$$TT_3f = 2.13\ \exp(-1.2t) \text{ and}$$

$$TT_3s = 3.67D\ \exp(-0.6t) - 2.13D\ \exp(-1.2t)$$

During this phase

$$E = C[11.11D\ \exp(-0.2t) - 2.13D\ \exp(-1.2t)]$$

The moment that $k_6TT_3s = k_5TT_3f$ occurs after $t = 2.34$ days. From this moment there is no net flow between both T_3 pools, therefore:

$$TT_3f = 0.130\ D\ \exp(-0.6t) \text{ and}$$

$$E = C[7.15D\ \exp(-0.2t) - 0.325D\ \exp(-0.6t)]$$

With an absorption percentage of 95% and 50 μg doses,

$$E = C[339.6\ \exp(-0.2t) - 15.44\ \exp(-0.6t)]$$

The normal value of $E = 135C$ is reached when $t = 4.5$ days. As a consequence, $(0.17 + 2.34 + 4.5)$ or about 7 days pass after the last T_3 dose has been taken before the TSH secretion resumes. The changes are depicted in figure 2-1. Also the actually measured TSH changes (320) are given, assuming that the maximal TSH level to be reached (AN/k_1) is 125 μU/ml. The TSH values, plotted as the difference between the actual level and this maximal level, perfectly follow the predicted decrease of the feedback inhibition. At the moment that the TSH secretion resumes, the model predicts the fast T_3 pool to be 0.41 μg or less than 2% of the amount in normals.

b. *Thyroxine medication stop*

After an abrupt stop of the T_4 substitution, the T_4 pool slowly decreases. Therefore the peripheral T_3 production will only diminish

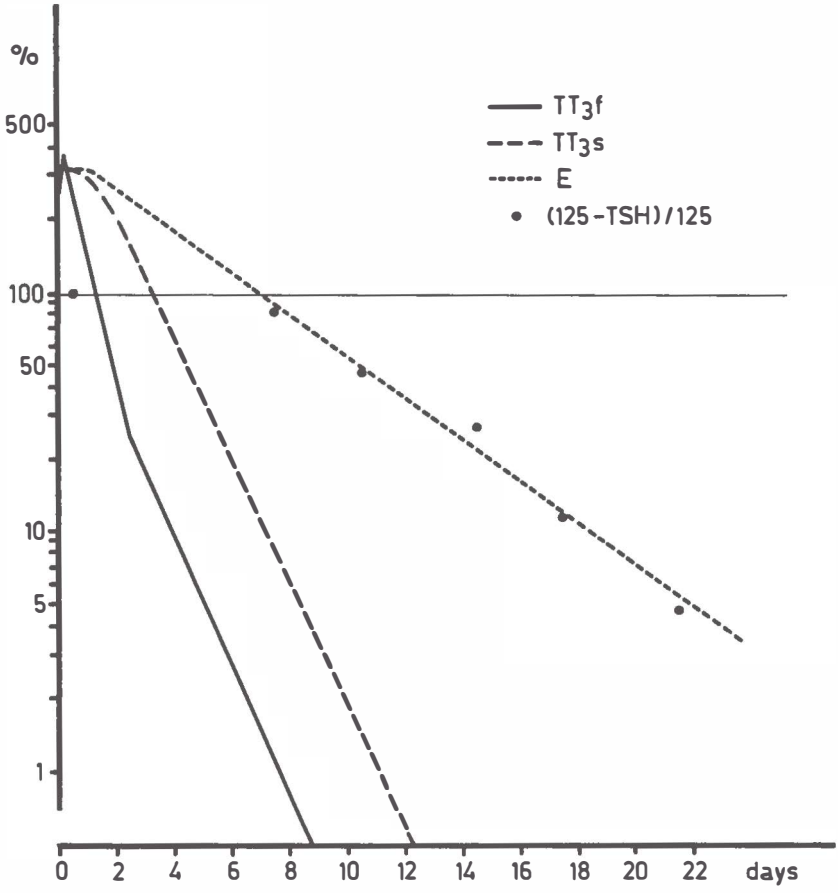


Figure 2-1. Predicted changes after stopping a regimen of 50 μg T_3 twice a day in thyroidectomized patients.
All parameters are represented in relation to the normal average (= 100%). The last dose is taken at time 0. The differences between the actually measured TSH values and the final maximum TSH value (= 125 μU/ml) are indicated. (normal average values: TT_3f = 27 μg TT_3s = 23 μg.)

gradually and it can be assumed that the slow and fast pools of T_3 stay in equilibrium. This means that at any time $k_5 TT_3f = (k_4+k_6)TT_3s$

and that the fractional disappearance in both pools is the same. Accordingly both pools can be taken together to form one single compartment.

With a single dose D per day, the changes occurring from the first day of the medication stop are:

$$TT_4 = (D/0.12)\ \exp(-0.12t) \text{ and}$$

$$TT_3 = -0.104D\ \exp(-0.6t) + 0.521D\ \exp(-0.12t)$$

This leads to a decrease in hormone effect

$$E = C[-2.531D\ \exp(-0.2t) + 0.140D\ \exp(-0.6t) + 3.517D\ \exp(-0.12t)].$$

With a regimen of 300 μg T_4 per day and an absorption percentage of 75% the hormone effect becomes after the stop:

$$E = C[-569.48\ \exp(-0.2t) + 31.50\ \exp(-0.6t) + 791.33\ \exp(-0.12t)]$$

The interval after which E reaches its normal value of 135C can be calculated to be 12 days. At that moment the model predicts a T_4 level amounting to 62% of the normal average, and a T_3 level of 56% of its normal average.

This is exactly what has been observed in normals: after one week the TRH-test is still suppressed, but has normalized after two weeks (329). A similar long interval of TSH suppression can be observed after a phase of subacute thyroiditis (330).

2.3.3 *Suppressive action of a single oral dose in normals*

a. *A single T_3 dose*

Let us assume that the secretions of TSH and thyroid hormone stop at the moment of ingestion of the dose. Taking a constant absorption completed in 4 hours and a constant flow from the slow pool to the fast pool, we can calculate the average changes in normals:

$$TT_4 = 717\ \exp(-0.12t)$$

$$TT_3f = (5.16\text{-}5D)\exp(-1.2t) + 19.92\ \exp(-0.12t) + 5D + 1.92$$

during the 4 hours of absorption and afterwards

$$TT_3f = (4.23+0.905D)\ \exp(-1.2t) + 19.52\ \exp(-0.12t) + 1.92).$$

During absorption and afterwards, the hormone effects becomes respectively:

$$E = C[(-118.44-30D)\exp(-0.2t)-(5.16-5D)\exp(-1.2t) + 249\exp(-0.12t) + 25D + 9.6]$$

and

$$E = C[(-110.63-1.002D)\exp(-0.2t)-(4.23+0.905D)\exp(-1.2t) + 244\exp(-0.12t) + 9.6]$$

The maximal value of E is reached when $dE/dt = 0$ and at that moment the TSH will be suppressed maximally. By the time that E has declined to its normal value of 135C, the TRH-test is normal again. With an absorption percentage of 95% we can calculate both intervals for different doses.

In case of a 50 μg dose the periods turn out to be 26 hours and 3.8 days. This is in accordance with the available data (321,327), suggesting that the effect is hardly noticable after 1 hour, is maximal after one day and has not yet returned to normal after 2 days. After one day the TSH response is not absent, but reduced to 30% (327). This indicates that the maximal effect, calculated to be 119% of its original value, is not high enough to blunt the TSH response completely.

For a 100 μg dose we calculate intervals of 36 hours for the maximal effect and 5.5 days for the normalization. It has been observed that the effect was maximal between 1 and 3 days and had returned to normal before 7 days (331). The TSH response was almost absent between 16 hours and 3 days, suggesting that with a 100 μg dose the effect is capable of blunting the response completely. The effect was calculated to be after 1 day, 36 hours and 3 days: 132, 139 and 127% of its original value respectively. The maximal effect after a 50 μg dose of 119% resulted in a 70% reduction of the TSH response, therefore the effect must be about 125% of the hormonal value to results in a complete suppression of the TSH response.

Also the T_3 levels are predicted well by the model. In case of a 100 μg dose the levels at 16 hours and 24 hours were calculated to be 259 and 218% of their original value, whereas levels of 251 and 218% were actually measured (331).

b. *A single dose of T_4*

Again it is assumed that the secretions of TSH and thyroid hormone stop at the moment the dose is taken. We take a constant absorption completed in 4 hours and assume a constant flow from the slow pool of T_3 to the fast pool, equal to the basal situation. For the period of absorption and the period afterwards we find:

$$TT_4 = (717\text{-}50D)\ \exp(-0.12t) + 50D \text{ and}$$

$$TT_4 = (702.66 + D)\ \exp(-0.12t)$$

$$TT_3f = (5.17 + 0.139D)\ \exp(-1.2t) + 0.028\ (717\text{-}50D)\ \exp(-0.12t) + 1.25D + 1.92 \text{ and}$$

$$TT_3f = (4.23\text{-}0.025D)\ \exp(-1.2t) + 0.028\ (702.66+D)\exp(-0.12t) + 1.92$$

$$E=C[(-118.38+11.252D)\exp(-0.2t)-(5.17+0.139D)\exp(-1.2t)+(248.96\text{-}17.363D)\exp(-0.12t) +6.25D+9.59] \text{ and}$$

$$E=C[(-114.45\text{-}0.380D)\exp(-0.2t)-(4.23\text{-}0.025D)\exp(-1.2t)+(244.0+0.35D)\exp(-0.12t)+9.59]$$

For a 3 mg dose with an absorption percentage of 50% (322) the effect becomes after the absorption period:

$$E = C[(-684.45)\exp(-0.2t)+33.87\ \exp(-1.2t)+ 769.0\ \exp(-0.12t) +9.59]$$

This means that 1, 2 and 3 days after the dose was taken, the effect E is 103, 116 and 126% of its normal value.

This is compatible with the observed decrease of the TSH response, being sharply reduced after 50 hours and completely absent after 74 hours (332). The maximal effect can be calculated to be 133% and is reached after about 5.5 days. The TRH-test can be predicted to normalize after 12 days.

The model further predicts T_4 levels at 1, 2 and 3 days of 278, 247 and 219% of the initial level. The actually measured values were 289, 256 and 216% respectively (332). At the same intervals the model predicts T_3 levels of 167, 176 and 165%, but the actually measured values were 169, 161 and 146% respectively (332). This indicates that the disappearance rate of T_3 from the fast pool gradually increases, probably through enhanced renal and fecal clearance.

2.4 CONCLUSIONS AND DISCUSSION

With the acceptance of a long-living messenger of the thyroid hormone effect in the pituitary, it is possible to describe the kinetic aspects of the complete thyroid hormone regulation system by means

of a rather simple direct proportional model. Sophisticated models lacking the hormone effect messenger have to be equipped with some sort of a memory within the pituitary, to account for the past history of the thyroid hormone levels. Therefore these models act by proportional as well as by integral control (319).

One important observation was made by means of such a model: any intrinsic activity of T_4 in the feedback mechanism can be omitted as long as the integral control of T_3, mimicking the messenger, is applied (319). Still some controversy exists on this matter. One of the main arguments for the acceptance of a role of intrinsic activity of T_4 in the feedback mechanism is the negative relation in normals between the serum T_4 and the TSH response to TRH. With the proposed model this can be explained. In the model a low serum T_4 level is equivalent with a low thyroidal amplification factor B. This in turn would mean a loss of loop gain. Therefore the control system would have to accept a large error signal, which means a low hormone effect, because

$$E/(N-E) = ABC\ (b + k_3/(k_2+k_3))(k_4+k_6)/(k_4+k_5+k_6)k_1k_4k_7$$

But, though B is low, there exists no clinical hypothyroidism, as the control system adapts itself. In an attempt to preserve the loop gain the number of thyrotropic cells is raised, resulting in slightly higher values of A (and M). This slight enlargement is recognized in the TRH-test by a higher TSH response. Such an adaptive reaction of the pituitary certainly restricts the fall in loop gain, but would lead to TSH dissipation when no further adaption occurred. Therefore the rise of the TSH level provokes a second adaptation: preferential T_3 secretion. In this way the drop of B is partly masked by a rise of b. The combination of these two adaptations will prevent a failure of the control system, at the expense of a relatively small rise of the TSH disposal.

With this interpretation we can properly explain the relations between serum T_4 and basal TSH, between serum T_4 and TSH response and between basal TSH and TSH response. In addition to this, the absence of a relation between serum T_3 and TSH response

becomes obvious, as well as the strongly positive relation between the serum T_3/T_4 ratio and the TSH response.

Though the proper substitution doses can also be predicted by models lacking the hormone effect messenger, these models fail in predicting the lag phases between a medication stop and the resumption of TSH secretion. The proposed model correctly predicts very long intervals of TSH suppression with subnormal levels of the thyroid hormones at the end of these intervals, after stopping oversubstitution with T_3 and T_4. Therefore one should not explain these lag phases by the assumption of a delayed adjustment of the pituitary (330) or some sort of recovery (329). Probably only after decades of oversubstitution leading to atrophy of the thyrotrophs some real recovery phase can be observed (329).

We initially accepted that the model would be unable to predict the levels exactly, because it was equipped with constant removal rates and constant volumes. But, as is shown, the model is not only applicable for qualitative changes, but also predicts quantitative changes sufficiently. When large elevations of the T_3 level are provoked however, the model is bound to predict the T_3 level to high, as the T_3 disappearance rate from the fast pool will rise markedly in these situations.

When studying different groups of thyroid patients, the model can be useful to explain the observed changes during dynamic tests in relation to the abnormality responsible for the clinical state of the patient. It might be possible to detect secondary changes in the control system, which attempt to mask the original failure. Therefore the model will be applied in the studies in the following chapters.

For convenience of the reader some of the parameters of the model, frequently returning in the following chapters, are compiled on a fold-out sheet at the end of the book. The symbols used for the parameters, and their meaning, are in this way always at hand.

Chapter 3

METHODS

3.1 NORMAL PERSONS AND PATIENTS

All individuals were tested by means of intravenous administration of synthetic TRH.

The normals studied by TRH injection were young healthy male volunteers, all hospital personnel or students. No one had a personal or family history of diseases known to interfere with the thyroid hormone control system. The "normals" studied by TRH infusion were referred to the hospital for medical reasons not connected with thyroidal or pituitary disease. Their condition was good; they did not suffer from febrile illnesses or severe chronical diseases. None showed a personal or family history of interfering diseases.

The patients studied had been referred to the hospital because of various thyroidal or pituitary diseases. They were in good general condition. Of the patients studied by TRH infusion, some data were reported before (333). These patients are grouped in a different manner for this study.

The patients were divided according to the following categories:

a. *Thyroid autonomy*

These patients showed either evident clinical hyperthyroidism with elevated free thyroxine levels ($FT_4F > 7$) and raised thyroidal uptakes (6 hours $> 38\%$), or a negative T_3 suppression test. Some of the patients with a negative suppression test, were clinically euthyroid or only marginally hyperthyroid; their free thyroxine levels ran parallel to the clinical impression of the metabolic state. Patients in the hyperthyroid phase of subacute thyroiditis were not included.

b. *Primary hypothyroidism*

This group of patients showed clinical hypothyroidism and low free thyroxine values ($FT_4F < 1.6$). It was not uniform with regards to the cause of the hypothyroidism and the duration of symptoms. In some of the patients the hypothyroidism occurred shortly after surgery or ^{131}I-treatment, in others the hypothyroidism developed gradually after ^{131}I-treatment or because of Hashimoto's disease.

c. *Preclinical primary hypothyroidism*

In these three patients vague complaints suggestive of hypothyroidism were present. The basal level of TSH was elevated (>6.5 μU/ml). In two patients the free thyroxine level was within normal limits (1.4 - 4.6). In the remaining patient the free thyroxine level could not be calculated, but the basal TSH level was only slightly elevated (9.0 μU/ml).

d. *Hypothalamic hypothyroidism*

The two patients studied were mildly hypothyroid and showed normal basal TSH values. The response of TSH to TRH was within normal limits. There was no pituitary enlargement and no evidence of pituitary neoplasm. Both patients showed a growth hormone deficiency. In one hypocorticism and hypogonadism were present.

e. *Abnormalities of the pituitary*

Patients with either endocrine hypo- or hyperfunction were included in this group. Some were hypothyroid, others euthyroid. The duration of symptoms in this group varied widely.

In the appropriate chapters more detailed data on the patients will be provided.

3.2 BLOOD SAMPLING AND STORAGE

Venous blood was sampled at appropriate intervals during the tests for several in vitro assays. The samples were drawn after as little as possible venous compression, while the person to be tested was in horizontal position during the whole procedure, to avoid interference with the assay results (334,335).

After clotting and centrifugation of the blood, each serum sample was split up into a number of subsamples. Subsamples for the assay of TSH and T_3 were stored at once at -20° C, the others were stored at 4°C until analysis within one week. Samples of one patient were always analyzed in the same assay run.

3.3 TRH ADMINISTRATION

All tests were started between 8.00 and 9.00 a.m. after the person to be studied had been in horizontal position for at least 30 minutes. In the TRH injection studies 200 μg or 500 μg of synthetic TRH (Hoechst) was injected intravenously as quick as possible. In the TRH infusion studies 1000 μg of synthetic TRH was infused in exactly 4 hours by means of an infusion pump (333).

3.4 LABORATORY METHODS

3.4.1 *Assay of thyroxine* (7.77 μg/100 ml = 100 nmol/L)

Thyroxine was measured in an ethanolic extract of the serum samples by means of a competitive protein binding technique. Commercial chemicals were used (Tetrasorb-125,Abbott) and the manufacturer's manual was followed. The normal range, excluding TBG abnormalities, for our hospital is 6.0 - 12.4 μg/100 ml (212). The intra-assay repeatability and the inter-assay reproducibility were similar: the coëfficients of variation were below 5% in the normal range. The measurements were performed by the Central Isotope Laboratory (head Prof.Dr.M.G. Woldring).

3.4.2 *The resin uptake test*

The uptake of ^{125}I-T_3 by resin in competition with the serum binding proteins was measured using a commercial kit (Triosorb-125,Abbott). The normal range, excluding TBG-abnormalities, is 23.2 - 37.6% (212). The intra-assay repeatability and interassay reproducibility showed coëfficients of variation below 5% in the normal range. The measurements were performed by the Central Isotope Laboratory.

3.4.3 *Assay of triiodothyronine* (65 ng/100 ml = 1 nmol/L)

Triiodothyronine was measured in unextracted serum by means of a radioimmunoassay, using the chemicals of a commercial kit (T_3-RIA,Abbott). The manufacturer's manual was modified, because the "blank binding" (binding of tracer without antiserum) of the standard samples markedly differed from the serum samples. Moreover the variation of "blank binding" of different sera was too large to neglect. Therefore the "blank binding" was measured in all samples.

Each kit contained:

5.5 ml T_3 labelled with ^{125}I (spec.act. 500-700 microCi/μg)
5.5 ml rabbit antiserum against T_3
2.0 ml standard T_3 (8 ng/ml)
3.0 ml serum devoid of T_3
2.5 ml charcoal-dextran suspension and
0.5 M borate buffer, pH=8.6 with 0.5% bovine serum albumen and 0.15% ANS (8-anilino-napthalene-1-sulphonate) to block the serum binding proteins.

Standard samples were made by dilution with the buffer. Before use, the charcoal-dextran suspension was diluted with 10 ml buffer, the T_3-less serum with 3.0 ml buffer. Each sample was run in duplo with added antiserum and in duplo without antiserum.

The standard vessels contained:

0.4 ml buffer, 0.1 ml T_3-less serum, 0.05 ml (standard) sample, 0.05 ml tracer and 0.05 ml antiserum or 0.05 ml buffer.

The serum vessels contained 0.1 ml buffer instead of the T_3-less serum.

After mixing, a 48 hours' incubation at 4°C was started. The separation of "bound" and "free" radioactivity was performed at 0°C (melting ice) in groups of 36 vessels. To each vessel 0.1 ml charcoal-dextran suspension was added, followed by mixing. After exactly 20 minutes the vessels were centrifuged for 10 minutes. The supernatant was separated from the precipitate by decantation. Both supernatant and precipitate were counted for radioactivity in a well type gamma counter. All radioactivity measurements were corrected for background. The total binding and blank binding were calculated as the ratio between the counted radioactivity in the supernatant and the total counts of supernatant and precipitate. The difference between total and blank binding was considered to be due to antibody-binding.

With a high affinity antiserum the binding percentage is C/(C+F+K), when C is the binding capacity of the antiserum, F is the amount of unbound antigen and K is the dissociation equilibrium constant. The mean binding percentages of 20 consecutive standard curves are given in table 3-1. From these data, showing good reproducibility, it can be calculated that K is small enough to be neglected. We calculated C to be 90 ng/100 ml (in the original undiluted serum), which means that without addition already 140 ng/100 ml of T_3 is present. By addition of extra amounts of tracer, we calculated that about 50 ng/100 ml of these 140 ng/100 ml originated from the added tracer T_3.

TABLE 3-1. Binding percentages of standard samples in 20 consecutive assay runs.

T_3 ng/dl	% binding	SD
0	62.0	1.54
50	46.4	2.18
100	37.2	2.27
200	26.4	1.86
400	17.0	1.59
800	9.8	1.09

The limit of detection was about 20 ng/100 ml. The repeatability and reproducibility showed coëfficients of variation below 5% for levels above 100 ng/100 ml. Normal values are 70 - 180 ng/100 ml.

3.4.4 *Assay of TSH*

The TSH levels in serum were measured by radioimmunoassay, as reported earlier (336). Normal values are <0.5 - 6.5 μU/ml. The assays were performed by the Central Isotope Laboratory.

3.4.5 *Other laboratory determinations*

Antibodies against thyroglobulin were detected by the tanned red cell haemagglutination technique, antibodies against thyroid cytoplasm or colloid by immunofluorescence (Streeklaboratorium van de Volksgezondheid, Groningen; Dr. J.A.M. Snyder). Thyroid uptake (RAIU) tests were performed by the Central Isotope Laboratory after oral administration of 5 or 50 microCi of ^{131}I. Thyroid scans were made by the Central Isotope Laboratory. Serum protein-bound iodine (PBI) was determined by the Central Laboratory (head Dr. A. Groen).

3.4.6 *Parameters of the free serum hormones*

Combination of the determinations of serum T_4 and resin uptake (RU) provides us a parameter of the free T_4 level (212). In order to correct for the differences of binding by TBPA of T_4 and T_3 we used as parameter:

$$FT_4F = T_4 \times RU/(1\text{-}0.6RU).$$

This parameter has been reported to correlate closely with the actual free T_4 level (208).

A similar combination, without TBPA correction, yields us a parameter of the free T_3 level:

$$FT_3F = T_3 \times RU.$$

Such a parameter, using talc uptake instead of resin uptake, has been reported to correlate closely with the actual free T_3 level (337).

3.5 STATISTICAL METHODS

Means, standard deviations and correlation coëfficients were calculated by usual methods. In the figures the significant correlations are expressed by lines calculated by the least square method. For comparisons Wilcoxon's test has been used. Differences and correlations will be called significant when the calculated p-value is below 5%. In the tables and figures only such p-values are indicated.

Chapter 4

TRH-INFUSION STUDIES

4.1 THE AIM OF THE STUDIES

The response of serum TSH to a bolus injection of synthetic TRH in normals and thyroid patients has been studied extensively by several investigators. Data on the response of serum TSH to a prolonged stimulus of TRH, by means of constant infusion, are scarce on the contrary. The responses of the serum thyroid hormones to exogenous TSH (mostly of bovine origin) have also been sufficiently studied. But little is known about the TSH response to infused TRH and the concomitant responses of the thyroid hormones.

The first aim of the infusion studies was to record in normals the responses of TSH, T_4 and T_3 to a prolonged TRH stimulus in order to become informed on the interrelationships between these responses. The second aim was to extend the same investigations to patients with a control system that shows an impending or evident failure, in order to study the changes of the control system in relation to the knowledge gathered in the normals. To get an idea about the periods of time needed for these adaptations the patients were grouped only according to their similar clinical state. Within a group the patients were deliberately selected for inhomogeneity regarding the cause of their clinical state and the time elapsed between the onset and the study. As a consequence no uniform outcome can be expected for either group and therefore the cases have to be discussed separately. At most some general remarks can be made in the end on the possibilities and limitations of self-correction of the control system.

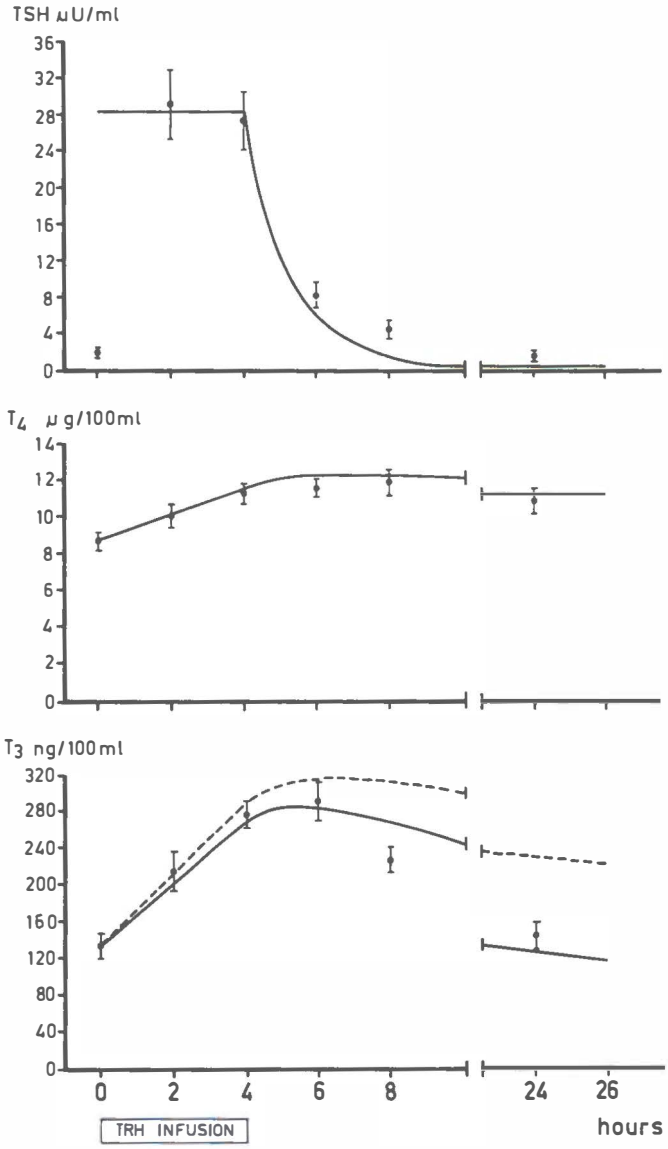


Figure 4-1. Mean levels (± 1 sem) of TSH, T_4 and T_3 in normals during and after a 4 hours' infusion of 1000 μg TRH. The changes predicted by the model are given by the lines. A constant level of TSH during infusion is assumed.
Solid line: $k_3 = 0$, interrupted line $k_3 = 0.03$

TABLE 4-1. Probability values of the changes found in normals during a 4 hours' infusion of 1000 µg TRH. (Wilcoxon's paired test, two-tailed; decreases in italics).

Difference	T_4	T_3	RU	TSH	FT_4F	FT_3F
0 h vs. 2 h	<0.01	<0.01		<0.01	<0.01	<0.01
0 h vs. 4 h	<0.01	<0.01	<0.02	<0.01	<0.01	<0.01
0 h vs. 6 h	<0.01	<0.01		<0.01	<0.01	<0.01
0 h vs. 8 h	<0.01	<0.01		<0.02	<0.01	<0.01
0 h vs. 24 h	<0.01				<0.01	<0.05
2 h vs. 4 h	<0.01	<0.02	<0.01		<0.01	<0.01
4 h vs. 6 h			*<0.02*	*<0.01*		
8 h vs. 24 h	*<0.02*	*<0.01*		*<0.01*	*<0.02*	*<0.01*

4.2 NORMAL PERSONS

The infusion of 1000 µg TRH was performed in ten normals. They were not selected for sex or age. The measured hormone levels and other relevant data are given in table A of the appendix. The mean levels (±SEM) are depicted in figure 4-1. The probability values of the changes of the parameters are given in table 4-1.

4.2.1 *Description of the changes in hormone levels*

The TRH stimulus provokes an elevation of the TSH level that is maintained until the end of the infusion at the same level as reached within two hours, indicating that there is no observable exhaustion. After the infusion has been stopped the TSH level rapidly falls, but does not reach its basal level within eight hours. After 24 hours the TSH level is not different from the starting value.

Due to the TSH elevation the levels of T_4 and T_3 rise during infusion, about linearly with time. In contrast to the fall of TSH the thyroid hormone levels are maintained for at least two hours after the infusion is stopped. Both thyroid hormone levels decrease from 8 to 24 hours, but only T_3 reaches its starting value by that time.

The changes of the resin uptake value are small, only at 4 hours a significant rise is observed.

The parameters for the free thyroid hormone levels, FT_4F and FT_3F follow the changes of the total hormone levels. In contrast to the total level, the free T_3 level at 24 hours is still above the starting value.

4.2.2 *Correlations of the responses*

As expected, according to data available in the literature (chapter 1), the highest TSH level reached is negatively correlated to the basal FT_4F ($r = -0.7283$ $p<0.05$), but not correlated to the basal FT_3F ($r = 0.2565$). A positive correlation is found between the highest TSH level and the ratio of basal FT_3F and basal FT_4F ($r = 0.6315$ $p<0.05$). No relation is found between basal FT_3F and FT_4F ($r = 0.2257$).

As a parameter of the pituitary amplification factor A we calculated the mean of the TSH rises at 2 and 4 hours ($= \Delta$ TSH). As shown in chapter 2 this value is equivalent to $A(N_1-N_0)/k_1$ as long as not all TRH receptor sites are occupied. From the final TSH values reached during constant infusion of amounts up to 25 μg TRH/min (62), that are much higher than the levels reached in this study, one can deduce that not all receptor sites are occupied during our infusion. This means that $(N_1 - N_0)$ will slightly differ with the variations in TRH distribution volume. These variations will be neglected in our calculations.

As parameters of the thyroid amplification factors B and bB, we calculated the apparent hormone secretion rates during infusion. Just as for the distribution volume of TRH we neglect variations in the distribution volumes of T_4 and T_3, taking volumes of V_4 and V_3f of 11 L and 22 L. When we express B and bB as μg hormone produced per day per μU TSH/ml, we find:

$$B = 330\ (2[T_4]_2 + [T_4]_4 - 3[T_4]_0)/\Delta\ TSH$$
$$bB = 0.66\ (2[T_3]_2 + [T_3]_4 - 3[T_3]_0)/\Delta\ TSH$$

The ratio b of the secretions of T_3 and T_4 can simply be calculated from these rates.

TABLE 4-2. Parameters for the functional capacities of the pituitary and the thyroid in normals, calculated from the data obtained by TRH infusion.

	ΔTSH	rise of T_3	rise of T_4	bB	B	b	$\frac{100\ \Delta FT_4F}{\Delta TSH}$	$\frac{100\ \Delta FT_3F}{\Delta TSH}$
	μU/ml	ng/dl per h	μg/dl per h	$\mu gT_3/d$ per μU/ml	$\mu gT_4/d$ per μU/ml		μg/μU	μg/μU
Son.	23.7	44.4	0.18	9.9	19.5	0.51	5.9	2.5
Han.	26.4	41.3	1.05	8.3	105.0	0.08	4.1	1.7
Verh.	20.0	23.1	0.49	6.1	64.4	0.10	5.0	1.4
Schel.	20.8	43.8	0.56	11.1	71.4	0.16	8.3	3.5
Band.	28.2	46.3	0.56	8.7	52.7	0.16	5.7	2.2
Vd Vl.	22.9	51.9	0.90	12.0	103.8	0.12	7.7	2.2
De Jag.	49.2	36.3	0.49	3.9	26.2	0.15	1.6	1.4
Uitd.	20.3	36.3	0.68	9.4	87.8	0.11	6.3	2.6
Sla.	15.6	11.3	0.66	3.8	112.1	0.03	10.1	1.2
Post.	37.3	41.9	0.81	5.9	57.5	0.10	7.0	1.5
Mean	26.4	37.7	0.64	7.9	70.0	0.15		
Median	23.3	41.6	0.61	8.5	67.9	0.11		

The values of these calculated parameters are given in table 4-2. It is evident that the amplification factors of the pituitary and the thyroid and the ratio of secretions of T_3 and T_4 do vary considerably even in normals. The median values of the secretions of T_3 and T_4 per μU/ml of TSH are close to the reported daily thyroidal production rates, indicating that the normal average basal TSH level is close to 1 μU/ml. The median basal level in these 10 persons is 1.55 μU/ml. The calculated median of b is close to the reported average normal value of about 0.10.

Though in the whole group the resin uptake values are rather constant, individual variations do occur and influence the individual values of b, bB and B. A good example of this phenomenon is person Son., where the total capacity of the binding proteins falls during the infusion. Whether this must be explained by a real shift of binding proteins from the serum or by affinity changes is uncertain.

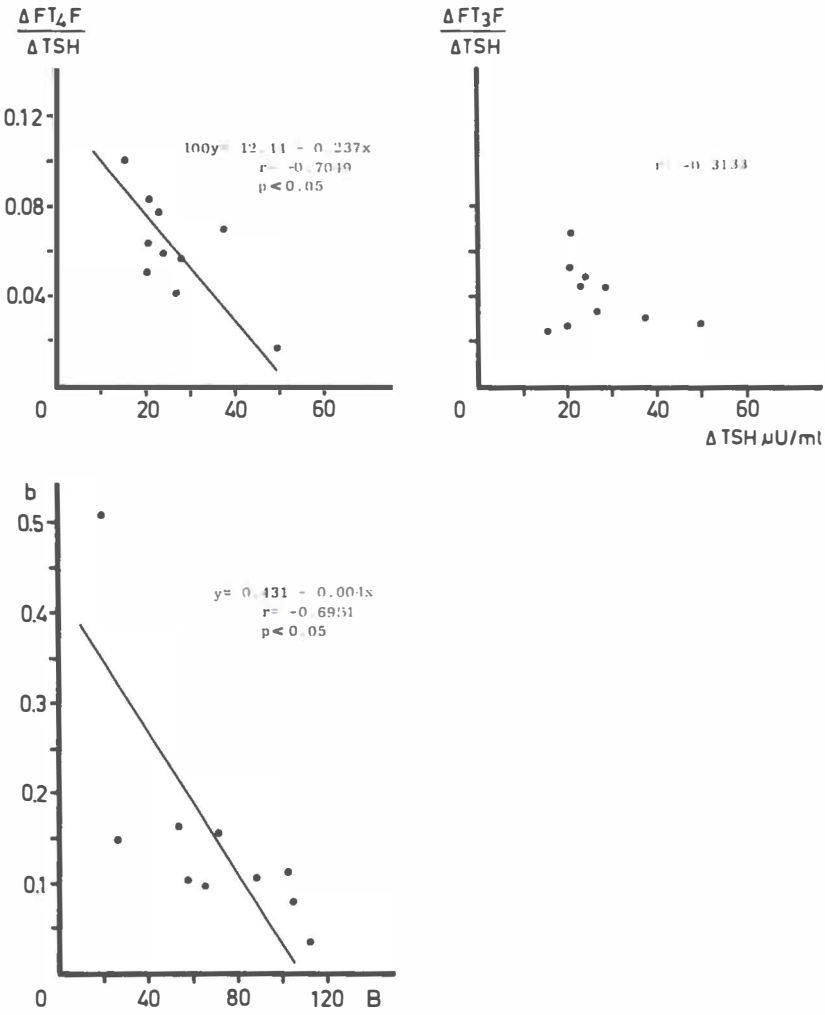


Figure 4-2. Relations in normals.
a) Upper part left: the relation between T_4-secretion per unit of TSH and the TSH response.
b) Upper part right: the relation between T_3-secretion per unit of TSH and the TSH response.
c) Lower part: the relation between the secretion ratio b of T_3 and T_4 and the T_4-secretion per unit of TSH (B).
$\Delta\ FT_4F = FT_4F(_4) - FT_4F(_0)$, $\Delta\ FT_3F = FT_3F(_4) - FT_3F(_0)$.
For Δ TSH, b and B see text.

In order to study the relation between the amplification factors A, B and bB, we can correct for these individuals resin uptake changes by taking the rises of FT_4F and FT_3F per μU/ml of TSH during the infusion instead of B and bB (table 4-2). As shown in figure 4-2a, the free T_4 response (Δ FT_4F) per unit of TSH is negatively correlated to the pituitary amplification factor A, measured as Δ TSH ($r = -0.7049$). In contrast, the free T_3 response (Δ FT_3F) per unit of TSH does not show such a relation (figure 4-2b, $r = 0.3133$). The reason for this is evident, as b is negatively correlated to B (figure 4-2c, $r = -0.6951$).

4.2.3 *Substitution of the parameters in the model*

When we neglect the individual variations of the distribution volumes by taking V_4=1L, and V_3f=22 L and V_3s=18 L we can substitute the values of table 4-2 in the model. Let us take bB = 0.33 μg/h per μU/ml of TSH and B = 2.92 μg/h per μU/ml of TSH, with a constant TSH level (Sp) during the whole infusion of 28.3 μU/ml.

After completion of the infusion the TSH level will drop according to $S=Sp \exp(-19.2t)$, assuming that the TSH secretion acutely stops. In this way, we calculate TSH level at 6, 8 and 24 hours of 5.7, 1.2 and <0.5 μU/ml, whereas the actual average values were 8.25, 4.32 and 1.52 μU/ml (fig 4-1). Besides the 24 hours' value,which is subject to considerable technical error, the 6 and 8 hours' values are above the predicted values. This indicates that the TSH secretion does not stop abruptly, but gradually decreases along with the occupancy of TRH receptor sites. In the further calculations however, we will neglect these differences.

During the infusion the total T_4 pool follows

$$TT_4 = -15571 \exp(-0.12t) + 16527$$

and afterwards

$$TT_4 = 1368 \exp(-0.12t) - 104 \exp(-19.2t).$$

These curves are shown in figure 4-1 by the solid line. It can be noticed that the values found are well predicted by the model.

Similarly we calculate the fast T_3 pool to be

$$TT_3f = -140.17 \exp(-1.2t) - 432.53 \exp(-0.12t) + 601.96 \text{ and}$$

$$TT_3f = 35.51 \exp(-1.2t) + 38.00 \exp(-0.12t) - 12.38 \exp(-19.2t) + 2.00$$

when we take $k_3 = 0.03$ and a fixed flow from the slow T_3 pool to the fast one equal to that in the basal state. These curves are shown in figure 4-1 by the interrupted line. Apparently the predicted values are far too high , from the moment that the T_3 secretion has stopped. Especially the fact that the T_3 level merely returns to its initial level, in face of a considerably increased total T_4 pool, is in conflict with the model prediction.

However, when we assume that from the beginning of the infusion the peripheral T_3 production stops abruptly, we calculate the fast T_3 pool to be

$$TT_3f = -159.52 \exp(-1.2t) + 188.78 \text{ and}$$

$$TT_3f = 68.63 \exp(-1.2t) - 12.45 \exp(-19.2t) + 2.00.$$

These curves, indicated by the solid line in figure 4-1, show a better fit to the observed levels. As argued before, the observed change of the T_3 level after the thyroidal secretion has stopped, occurs faster than predicted by the model (note the 8 hours' value). The observation that the 24 hours' value actually measured is higher than the predicted one, might indicate that the peripheral production of T_3 has resumed by that time.

To explain the sudden discontinuation of peripheral T_3 production two interpretations are open. One might suggest that the whole procedure of the infusion causes enough stress to the patients to provoke the mechanism responsible for the reduction of peripheral T_3 production along the lines of the glucocorticoid theory. On the other hand it can be suggested that the enhanced production of rT_3, due to the suddenly raised total T_4 pool, is insufficiently balanced by the further degradation of rT_3, leading to an increase of the intracellular rT_3 level. This increase might be high enough to inhibit the T_3 production almost completely for a considerable period. The second interpretation seems unlikely however, as such a phenomenon was not observed after an oral dose of 3 mg T_4 (332).

The hormone effect E changes according to the following formulas, when we assume $k_3 = 0$:

$$E = 957.12 \exp(-0.2t) + 159.52 \exp(-1.2t) + 943.9 \text{ and}$$

$$E = 207.32 \exp(-0.2t) - 68.63 \exp(-1.2t) + 0.66 \exp(-19.2t) + 10.0.$$

This means that at 24 hours the hormone effect is 110% of its original value. The number of normals studied was too small however to detect a significant suppression of the TSH level.

4.3 THYROID AUTONOMY

The same TRH infusion procedure as in normals was performed in 20 patients with evident hyperthyroidism or an abnormal T_3-suppression test. Relevant data on these patients are provided in table 4-3 and the measured parameters are given in tables B and C of the appendix. It should be noted that the low T_3 level in patient Wie. most probably resulted from inhibition of peripheral T_3 production by the triple dose of oral contrast for cholecystography. The oral contrastmedia used for this radiographic investigation are known to interfere for some weeks (227).

4.3.1 *Description of measured responses to TRH-infusion*

As expected, there was no or only a minimal TSH response to the TRH administration. Likewise no response of T_4 or FT_4F was observable. The last patient (Bro.) of this group, was the exception to the rule: the responses of TSH, T_4 and T_3 were all clearly present. Probably the daily production by the autonomous part of her thyroid was about the daily need, which is compatible with the clinical impression.

Surprisingly enough, not only patient Bro. showed a T_3 response. In about half of the patients the rise of FT_3F was higher than the lowest response (19.2) in the normal group, despite the virtually absent TSH response.

In order to try to detect a cause for this remarkable response, the group was divided in "non-responders" ($\Delta FT_3F < 19.2$) and "responders" ($\Delta FT_3F > 19.2$). In the subgroup of "non-responders"

TABLE 4-3. Some data of the patients in the category "autonomy".(upper part the "non-responders", lower part the "responders".

	Age/sex	FT_4F	FT_3F	%RAIU(basal)		%RAIU(T_3suppr.)		thyroid
				6 h	30 h	6 h	30 h	scintigram
Pip.	56/M	10.04	96.6	50.8	56.7			H
And.	48/F	9.26	177.6	56.9	53.0			H
Goe.	50/F	8.00	139.3	64.5	63.8			H
Dou.	74/F	13.60	156.8	56.7	59.0			H
Wie.1)	56/F	7.89	27.0	28.2	52.6			MN
Jan.	51/F	8.40	77.5	38.5	48.5			H
Zui.	48/F	8.88	114.9	48.3	58.3			MN
Smi.	57/F	4.78	42.5	43.4	50.8	41.4	56.8	N
Beu.	54/F	3.15	38.1	25.4	37.2	20.9	38.0	MN
Lin.	66/F	2.99	61.0	47.5	53.4	44.6	53.6	MN
Van M.	64/F	4.55	210.6	28.0	36.4	20.4	30.4	N
Mul.	50/F	8.48	144.1	55.0	57.0			MN
Roo.	68/F	8.00	126.6	51.0	54.7			H
Van A.	20/M	8.62	114.0	50.0	60.2			H
De W.	56/F	10.07	129.9	46.0	54.5			H
Nic.	60/F	8.27	95.2	39.3	45.4			MN
Pou.	78/M	10.70	65.3	49.3	57.0			MN
Sto.	59/F	4.70	85.0	33.7	48.5	29.0	42.6	N
Bos.	69/F	6.11	64.4	36.1	41.4	31.9	38.7	N
Bro.	29/F	3.19	38.1	31.1	37.8	28.0	43.7	H

The scintigraphic impression of the thyroid is indicated by H = homogenous, MN = multinodular and N = uninodular.
1) received a single and a repeated double oral dose of iodinated contrastmedium for cholecystography,both within 3 weeks before the tests.

no responses were detectable of T_4, T_3, RU, FT_4F and FT_3F. At 2, 6 and 8 hours the TSH level was higher than the starting value (p values smaller than 0.05, 0.01 and 0.05 respectively), though the differences were very small (see table B of the appendix).

In the "responders" no evident responses were found of T_4, RU and FT_4F (only the T_4 levels of 2 and 4 hours differed, $p<0.05$). No rise of TSH could be observed. That the 8 hours' TSH level is above the starting value ($p<0.02$) can hardly be looked upon as a response to the infusion. Of course there is a response of T_3 and FT_3F in this subgroup.

Comparison of the basal values of T_4, T_3, RU, FT_4F, FT_3F, FT_3F/FT_4F and TSH did not reveal any difference between both subgroups. Furthermore there was no difference in thyroidal iodine uptake or age. Also the distribution over the subgroups of the sex and of the type of thyroid (H, N or MN) did not appear to be different.

4.3.2 *Calculation of the model parameters*

An attempt was made to calculate the parameters Δ TSH, bB and B, just as in the group of normals. The ratio's were only calculated when both terms of the ratio were positive. Because the technical error in Δ TSH will be high, the calculated values of bB and B give only the order of magnitude (table 4-4).

As expected, the values of Δ TSH, bB and B found in patient Bro. are similar to the values found in normals. However, in most of the remaining cases where bB and/or B were calculated, these values tend to exceed the normal values markedly, in the "responders" as well as in the "non-responders".

This means that at least a majority of the hyperfunctioning thyroid glands is extremely sensitive to stimulation. Probably the prolonged stimulation by TRH is capable of inducing a minor secretion of TSH, that is high enough in about half of the cases to be followed by a readily observable T_3 response of the oversensitive thyroid gland. The possibility can not be excluded, that the diseased glands do react to TRH instead of to TSH. As will be described in chapter 5, no evidence of this kind can be obtained however.

TABLE 4-4. Parameters of the functional capacity of the thyroid to respond to TSH in patients of the category of thyroid autonomy.(upper part:"non-responders", lower part:"responders").
N.B. The value of ΔTSH is not a parameter of the functional capacity of the thyrotropic cells, as the feedback inhibition by thyroid hormone exceeds the setpoint of the pituitary.

	ΔTSH	rise of T_3	rise of T_4	bB	B	b
	μU/ml	ng/dl per h	μg/dl per h	$\mu gT_3/d$ per μU/ml	$\mu gT_4/d$ per μU/ml	
Pip.	0.3	0.0	-0.24	0.0		
And.	0.0	0.0	-0.13			
Goe.	0.3	0.6	-0.09	11.0		
Dou.	2.3	11.9	-0.20	27.3		
Wie.	3.7	5.0	-0.49	7.1		
Jan.	1.0	-1.3	-0.10			
Zui.	1.6	9.4	0.41	30.9	680	0.05
Smi.	0.1	6.3	-0.25	330		
Beu.	0.2	7.5	-0.25	198		
Lin.	0.0	-10.6	-0.13			
Van M.	0.1	7.5	0.31	396	8250	0.05
Mul.	0.3	25.0	-0.16	440		
Roo.	2.6	27.5	-0.01	55.8		
Van A.	-0.3	28.8	-0.54			
De W.	1.1	27.5	-0.40	132		
Nic.	0.0	36.9	-0.21			
Pou.	0.6	21.3	-0.21	187		
Sto.	-0.2	73.8	0.01			
Bos.	-0.1	28.1	0.08			
Bro.	10.2	15.0	0.61	7.8	158.5	0.05

4.4 PRIMARY HYPOTHYROIDISM

Eight patients with clinically evident thyroid failure, due to different causes, were studied by TRH infusion. Some data on these patients are provided in table 4-5.

TABLE 4-5. Data of the patients in the category of primary hypothyroidism. (= failure originating in the thyroid).

	Age/sex	FT_4F	FT_3F	TSH	% RAIU 6 h	% RAIU 30 h	remarks
Vd. M.	39/F	1.58	30.3	19.5 (recent IVP)			thyroid antib. pos.
Col.	68/F	1.33	31.3	45.1	10.8	21.8	thyroid antib. pos.
Mol.	53/F	1.26	29.6	77.9	17.3	25.9	^{131}I-treatm.,77 w. before
Ber.	35/F	1.23	20.9	32.5	1.7	3.7	thyroid antib. pos.
Sie.	43/F	0.35	2.2	36.0	1.3	2.2	^{131}I-treatm.,25 w. before
And.	49/F	0.83	20.1	19.0	9.0	16.8	^{131}I-treatm.,14 w. before
Tou.	43/M	0.63	14.6	40.0			thyroidect.,3 w. before
Sch.	45/F	0.64	4.2	35.1			thyroidect.,6 w. before

4.4.1 *Description of the measured responses*

The measured parameters during and after TRH infusion are compiled in table D of the appendix.

In all patients the basal level of T_4 is below the normal lower limit and the basal level of TSH is considerably above the normal upper limit.

The four patients with the lowest basal T_4 levels ($<2.5\ \mu g/100$ ml) showed the lowest basal T_3 levels as well. In these patients the onset of thyroid failure occurred abruptly and shortly before the study: patients Tou. and Sch. underwent total thyroidectomy 3 and 6 weeks before respectively, whereas patients Sie. and And. received a ^{131}I-therapy dose 25 and 14 weeks before respectively. The TSH response in these patients is relatively small and similar to the response in normals. In two of them there are even signs of exhaustion during the infusion: the TSH value at the end of the infusion has dropped to a level hardly higher than the starting value. No clear reaction of the serum T_4 or T_3 is noticeable.

The remaining patient with a post-^{131}I-therapy hypothyroidism received the last therapy dose 77 weeks before the test. The other three patients showed high thyroid antibody titers. In these four patients the basal T_3 level is in the normal range and their basal FT_4F is above 1.2. All show a large TSH response to the infused TRH and in all there is a T_3 response detectable.

TABLE 4-6. Calculated parameters of the functional capacities of the pituitary and the thyroid in the patients of the category of primary hypothyroidism.

	ΔTSH	rise of T_3	rise of T_4	bB	B	b
	μU/ml	ng/dl per h	μg/dl per h	μgT_3/d per μU/ml	μgT_4/d per μU/ml	
Vd M.	135.6	13.1	0.29	0.51	5.60	0.09
Col.	89.0	22.5	-0.43	1.33		
Mol.	56.7	19.4	0.10	1.80	4.66	0.39
Ber.	112.8	3.1	0.14	0.15	3.22	0.05
Sie.	9.9	0.0	-0.09	0		
And.	33.0	4.4	-0.01	0.70		
Tou.	12.2	0.0	-0.09	0		
Sch.	27.4	-2.5	-0.15			

4.4.2 *Calculation of the model parameters*

As in the other categories, the values of Δ TSH, bB, B and b were calculated; they are given in table 4-6. These data indicate the severe loss of functional capacity of the thyroid in all patients. The first four patients, in which the hypothyroid state is least severe, manage to keep their T_3 level in normal range solely because of an increased functional capacity of their thyrotropic cells. Probably the loss of thyroid function progressed slowly enough to facilitate pituitary growth.

4.5 PRECLINICAL PRIMARY HYPOTHYROIDISM

The three patients of this category had only some vague symptoms suggestive for hypothyroidism. Their thyroidal uptake values were higher than found in the category of primary hypothyroidism; in two of them the uptake can be considered to be increased. In two patients the total and free levels of T_4 and T_3 were within normal limits. In the other the total T_4 level was 5.0 μg/ml, but as this patient had

autoantibodies against T_3 the resin uptake value probably is spuriously low. This means that probably his free T_4 level was within normal limits as in the other two (some studies in this patient will be described in chapter 6). In all three the basal TSH level was above normal limits. Some data on these patients are given in table 4-7.

TABLE 4-7. Data of three patients with preclinical primary hypothyroidism (upper part) and of two patients with hypothalamic hypothyroidism (lower part).

	Age/sex	FT_4F	FT_3F	TSH	% RAIU		remarks
					6 h	30 h	
Bla.	53/F	2.16	39.8	11.0	19.9	30.0	^{131}I-treatm.,9 y. before
Ren.	20/F	2.73	42.7	18.8	38.6	46.7	thyroid antib. pos.
Lou.	15/M	-	-	9.0	51.8	66.2	thyroid antib. pos.
8re.	16/F	1.64	21.2	<0.5	13.8	19.9	GH-deficiency
Vee.	23/M	1.30	23.4	0.9	7.7	11.4	GH-deficiency, hypocorticism,hypogonadism

4.5.1 *Description of the changes during and after TRH infusion*

The measured parameters of these patients are given in table E of the appendix. Though the starting thyroid hormone values are higher than in the primary hypothyroid group, the responses in these patients are similar to those of the first four patients of the primary hypothyroid group. Especially the large response of TSH is remarkable.

4.5.2 *Calculated model parameters*

The calculated values of Δ TSH, bB, B and b are given in table 4-8. Obviously there is a large loss of thyroid capacity in these patients, which is similar to the situation found in clinically evident primary hypothyroidism. In all three patients the pituitary capacity is markedly increased.

4.6 HYPOTHALAMIC HYPOTHYROIDISM

Two patients with multiple deficiencies of pituitary hormones, including TSH deficiency, were tested by TRH infusion. Both showed a retarded bone age, in both the sella turcica was small. The thyroid hormone substitution had been stopped at least six weeks before the test. Patient Vee. was studied twice, with an interval of a month, in order to get an impression of the lag phase of the T_3 secretion in this condition. Data on the patients are given in table 4-7.

4.6.1 *Description of the responses to TRH infusion*

The measured parameters of these patients during the test are given in table F of the appendix. Upon the TRH infusion the TSH levels increase, however in all three tests there is evidence of exhaustion of the pituitary TSH stores. In both patients the thyroid clearly responds to the TSH rise.

TABLE 4-8. Calculated parameters of the functional capacities of the pituitary and the thyroid in the patients with preclinical primary hypothyroidism (upper part) and in the patients with hypothalamic hypothyroidism (lower part).

	ΔTSH	rise of T_3	rise of T_4	bB	B	b
	μU/ml	ng/dl per h	μg/dl per h	μgT_3/d per μU/ml	μgT_4/d per μU/ml	
Bla.	125.1	13.1	0.33	0.55	6.9	0.08
Ren.	129.7	17.5	0.25	0.71	5.1	0.14
Lou.	113.6	-	0.68	-	15.7	
Bre.	14.0	13.8	0.46	5.2	87.2	0.06
Vee. I	12.6	25.6	0.38	10.7	78.6	0.14
Vee.II	10.7	18.8	0.39	9.3	95.6	0.10

4.6.2 *Calculation of the model parameters*

The calculated model parameters are given in table 4-8. In both patients the values of bB and B are within the range found in normals,

indicating that there is no functional loss of the thyroid observable, when the stimulation is of some hours' duration. The value of Δ TSH is below the range found in normals, indicating that the number of thyrotrophs and the amount of stored TSH is low. This is compatible with the diagnosis of TRH deficiency. Judged from the 2 hours' TSH value, the initial response of the pituitary is normal in both patients. This means that both the pituitary and the thyroid are normally reactive in this condition. The deficiency of TRH results in a lower setpoint as well as in a diminished TSH store.

4.7 ABNORMALITIES OF THE PITUITARY

Six patients, of whom the data are given in table 4-9, were tested by TRH infusion. One of them, patient Zijl., was restudied 4 weeks after surgery, when he was still euthyroid clinically. Seven months after the operation he was hypothyroid however, and received treatment. In patient Boo. there was no radiological evidence of a pituitary abnormality. Other symptoms were strongly suggestive of a partial syndrome of Sheehan, following a sectio caesarea. After this event her diabetes could be regulated by 4-8 Units/day, in contrast to the 40 Units needed before. Signs of hypocorticism were present. A deficiency of GH and ACTH was confirmed biochemically.

TABLE 4-9. Data of the patients with pituitary abnormalities.

	Age/sex	FT_4F	FT_3F	TSH	pituitary abnormalities	remarks
Boo.	49/F	3.67	22.9	0.9	partial Sheehan's syndrome	euthyroidism,GH-def.,hypocortic.
Schi.	40/M	3.05	49.7	1.7	intrasellar tumor	euthyroidism,active acromegaly
Zijl.I	38/M	2.17	55.2	2.8	chromophob. ad. intra- and supra-sellar extension	euthyroidism
Zijl.II		2.30	29.6	0.6		euthyroidism, 4 w. after surgery
Tem.	45/M	2.52	41.4	<0.5	eosinophyl. ad. intrasellar loc.	euthyroidism, 11 mo. after surgery
Nij.	54/M	1.61	29.2	1.9	chromophob. ad. intra- and supra-sellar extension	hypothyr.,hypocortic., 3 y. after surgery
Sche.	36/F	1.23	28.2	0.7	hypophysect.	panhypopit., 2 w. after surgery

4.7.1 *Description of the responses to TRH infusion*

The measured data on these patients are given in table G of the appendix. In all patients there is a T_3 response, though in some of them this response is below that of normals. A low T_3 response is always accompanied by a low TSH response. There is no clear relation between the TSH response and the clinical state.

4.7.2 *Calculation of the model parameters*

The calculated model parameters are given in table 4-10. In the two euthyroid cases with an unoperated tumor (Schi. and Zijl. I) the responses of the pituitary and the thyroid can be considered normal. All other tests reveal a reduced pituitary capacity. Remarkable is, that in the two remaining stable euthyroid cases Boo. and Tem. the sensitivity of the thyroid to TSH appears to be increased. In patient Boo. the residual number of thyrotropic cells is probably so small that the amount of stored TSH is insufficient to maintain a constant TSH level during the infusion.

TABLE 4-10. Calculated parameters of the functional capacities of the pituitary and the thyroid in the patients with pituitary abnormalities.

	ΔTSH	rise of T_3	rise of T_4	bB	B	b
	μU/ml	ng/dl per h	μg/dl per h	μgT_3/d per μU/ml	μgT_4/d per μU/ml	
Boo.	1.5	20.0	0.19	70.4	330	0.21
Schi.	22.5	25.0	0.43	5.9	49.9	0.12
Zijl. I	14.2	11.9	0.70	4.4	130.1	0.03
Zijl.II	7.6	28.1	0.19	19.5	65.1	0.30
Tem.	3.7	15.0	0.34	21.4	241	0.09
Nij.	6.2	12.5	0.61	10.6	261	0.04
Sche.	-0.1	2.5	0			

Even in the hypothyroid patient Nij. the value of B exceeds that of the normals; in this case it is probably a deficiency of TRH that is responsible for the hypothyroid state. The second test of patient Zijl.

shows a shift from thyroidal T_4 production to T_3 production. This might indicate a reduction in iodine availability; indeed the thyroidal uptake (6h 11.5%, 30h 24.1%) was below the normal lower limits (6h 15%, 30h 25%). Probably TRH deficiency is also in this case the main cause for the developing hypothyroidism.

4.8 SUMMARY AND DISCUSSION

The infusion during four hours of synthetic TRH enabled us to investigate the response of the thyrotropic cells in combination with the subsequent response of the thyroid to endogenous TSH.

4.8.1 *The results in normals*

In normals the responses of TSH as well of the thyroid hormones vary considerably. From these responses model parameters can be calculated, that represent the sensitivities of the pituitary and the thyroid to their stimulators. Using these parameters, the response variations were studied further.

The relations between these calculated parameters suggest that the control system can adapt itself in order to keep its control function as optimal as possible, or, in other words, to keep the loop gain within certain limits. Accordingly, the thyrotropic part of the pituitary compensates a reduction in functional capacity of the thyroid by growth (i.e. B is inversely related to Δ TSH). As a consequence more TSH is secreted upon the same error signal, leading to a preferential secretion of T_3 (i.e. b is inversely related to B, but bB is independent of Δ TSH).

As not all T_4 is eventually converted to the active T_3, only a part of the hormonal potency of T_4 comes to expression. As shown in chapter 2, the gain of the thyroid in terms of hormone effect is expressed by $bB + Bk_3/(k_2+k_3) = bB + 0.25B$. This means that a parameter of the total loop gain is Δ TSH $(bB + 0.25B)$. The value of this parameter (TLG) in the normals is given in table 4-11.

The magnitude of TLG indicates the rate of secretion of hormonal potency expressed as μg T_3 per day, that is resulting from the combined responses of the pituitary and the thyroid to a constant infusion of 4 μg TRH per minute.

TABLE 4-11. Total loop gain (TLG = ΔTSH(bB + 0.25 B)) in various categories in terms of the production rate of hormonal potency, expressed as μg T_3/d, resulting from infusion of 4 μg TRH/minute.

normals		primary hypothyr.		preclin. primary hypothyr.		hypothal. hypothyr.		pituitary pathology	
Son.	351	Vd M.	259	Bla.	285	Bre.	378	Boo.	229
Han.	913	Col.	118	Ren.	258	Vee. I	383	Schi.	414
Verh.	444	Mol.	168	Lou	>446	Vee.II	355	Zijl. I	524
Schel.	603	Ber.	108					Zijl.II	272
Band.	618							Tem.	302
Vd Vl.	870	Sie.	0					Nij.	470
De Jag.	517	And.	23					Sche.	0
Uitd.	637	Tou.	0						
Sla.	496	Sch.	0						
Post.	757								

4.8.2 *The results in primary hypothyroidism*

The results in the primary hypothyroid patients indicate that the adaptive growth of the thyrotropic part of the pituitary takes considerable time. When hypothyroidism develops more or less abruptly through removal or irradiative destruction of functional thyroid capacity, as after thyroidectomy or in hypothyroidism developing within 6 months after ^{131}I-treatment, there is no evidence of any significant pituitary growth. The situation differs when the destruction of functional capacity of the thyroid is thought to proceed gradually, as in Hashimoto's disease and in the final stage of Graves' disease. In this situation the pituitary growth can cope with the thyroidal destruction during some interval and the complete failure of the control system does not become evident before the thyrotropic

capacity has been enlarged considerably. The extent of this kind of hyperplasia is far from insignificant. Enlargement of the sella turcica has been described in primary hypothyroidism (55).

The attempt of the pituitary to compensate a thyroid failure is also reflected in the TLG (table 4-11). In the "acute" cases, the TLG is less than 10 percent of normals. In the gradually developed cases the TLG is still considerable, though certainly lower than in normals; still, normal levels of T_3 can be maintained.

4.8.3 *Results in preclinical primary hypothyroidism*

The observations in the first two cases of preclinical primary hypothyroidism add further evidence for pituitary adaptation. Though their TLG is reduced (table 4-11), it is higher than in the clinical hypothyroid cases. Yet the thyroid capacity is reduced already to some 10 to 20% of normal. This indicates that the adaptive increase of the number of pituitary thyrotropic cells easily reaches a factor 5 before the failure of the thyroid becomes clinically observable, in case the rate of thyroidal destruction is low. This implicates that, when the destruction of the thyroid stops or is kept in balance by cell division, a perfectly normal daily thyroid hormone production can be maintained for years at the expense of a raised TSH secretion by an adaptively enlarged pituitary. In these cases the TLG can be normal, through a combination of a reduced thyroid gain and an increased pituitary gain. This phenomenon has been recognized in euthyroid goiter, Hashimoto's disease, after ^{131}I-therapy and after subtotal thyroidectomy (109,110,111,179,181). In euthyroid idiopathic goiter the number of functional thyroid cells may eventually increase through the sustained slight elevation of the basal TSH level to such an extent, that the elevated TSH level declines. Indeed the basal TSH level in euthyroid idiopathic goiter is higher during the first year of goiter development than afterwards (111).

In patient Lou., with circulating antibodies against T_3, the value of TLG can not be calculated. However, when the secretion of T_3 is omitted, a value of 446 (= Δ TSH. 0.25B) is found. This value is in

the normal range already and indicates that in this patient the TLG surely was normal. Yet the basal TSH was elevated because only a part of the produced hormonal potency eventually exerted its effect, as a great deal of the produced T_3 was neutralized by the antibodies. More details of this case will be provided in chapter 6.

4.8.4 *Results in hypothalamic hypothyroidism*

Both patients with a diagnosis of TRH deficiency show a normal thyroidal response to endogenous TSH. Therefore in these patients there is no evidence of functional loss of the thyroid or of the production of biologically less potent TSH, as suggested by some authors (107,203). In a repeated test in one of the patients additional samples were drawn at 10, 20, 30 and 40 minutes from the infusion start in order to determine the lag phase of the secretion of T_3. From the measured T_3 levels (table F appendix) it may be deduced that significant amounts of T_3 are only secreted after 30 minutes of stimulation. After a bolus injection of TRH the increase of the T_3 level in normals does not show such a lag phase, as the increase is about linear to the elapsed time for 120 minutes (107). Therefore the absence of a T_3 response to a TRH injection must not be interpreted as evidence for thyroid atrophy or abnormal TSH, but can be explained by a lag phase. This lag phase probably results from the unstimulated state of the thyroid at the beginning of the test. By the time the thyroid is activated during a TRH test the TSH value has dropped to almost basal levels already and the T_3 secretion will be delayed and low. When the TSH level is kept high as in our TRH infusion test, there will be a normal rise of T_3 after the lag phase has been passed.

The calculated TLG of both patients can be considered to be in the low normal range. Had the TLG been measured after a more physiological stimulus than 1000 μg TRH, it probably would have been normal, as Δ TSH would not have been influenced by exhaustion then. Therefore, also the thyrotropic cells seem to react normally to TRH as long as the TSH stores are not exhausted.

Accordingly, the abnormalities of the control system encountered in hypothalamic hypothyroidism are resulting directly from TRH deficiency and there is no evidence of fundamental changes in the function of both thyrotrophs and thyroid.

4.8.5 *Results in patients with pituitary abnormalities*

The results in the patients with pituitary abnormalities are most remarkable. The fact that all parameters, including the TLG (table 4-11), are similar to those in normals in the two euthyroid patients (Schi. and Zijl. I) with an unoperated tumor, is understandable. It simply means that the tumor did not significantly interfere with the thyroid control system. The other two stable euthyroid cases however (Boo. and Tem.) showed a drastically reduced pituitary capacity. Yet their thyroid hormone responses were normal, indicating that their thyroids secreted an increased amount of thyroid hormone per unit of TSH.

As the calculated TLG value of patient Boo. will be spuriously low because of TSH exhaustion during the test, the TLG values of these patients are comparable to the values found in preclinical hypothyroidism (table 4-11). This means that the control system is able to adapt itself sufficiently not only to partial thyroid failure but also to partial pituitary failure, by increasing the sensitivity of the thyroid to TSH. Judged from the values of bB and B (table 4-10), the sensitivity of the thyroid can be increased by at least a factor 2. Though the TLG is lower than in normals, it is high enough to ensure euthyroidism, just as in preclinical primary hypothyroidism. The preferential secretion of T_3 in patient Boo. can reflect the reduced availability of iodine to the thyroid, caused by the relative TSH deficiency.

Surgery in the region of hypothalamus and pituitary may lead to disruption of the transport route of TRH to the pituitary, resulting in a condition similar to idiopathic TRH deficiency. The results in patients Nij. and Zijl. II are suggestive of such a condition. Though the TSH responses were reduced, their TLG must be considered to be high enough to ensure stable euthyroidism. Yet patient Nij. was

evidently hypothyroid already and patient Zijl. had to be substituted soon afterwards.

Remarkable is the shift to preferential T_3 secretion in patient Zijl. after operation. Probably the TRH deficiency resulted in a change of thyroidal iodine uptake, leading to preferential T_3 production. At the time of the second test the 6 and 30 hours' uptake percentages were 11.5 and 24.1%, which dropped to 7.1 and 14.1% six months later when he had become hypohyroid. At that moment TRH infusion resulted in an increase of the TSH level to 18.6 μg/ml (333), indicating that TRH deficiency must have been the cause of the hypothyroid state.

The results in patient Sche. are in accordance with a status after total hypophysectomy.

4.8.6 *Results in patients with autonomously functioning thyroids*

Though several patients were clinically euthyroid and had normal basal T_3 levels, the lack of response of TSH to TRH infusion in almost all patients with an autonomous thyroid, is not surprising in view of the extreme sensitivity of the pituitary to thyroid hormone overproduction (116). Also the observation of a TSH response in one clinically euthyroid patient despite the absence of T_3 suppression, is hardly astonishing. It can be explained easily by accepting that the autonomously produced amount of thyroid hormones is equal to or below the daily needed production.

As expected, the T_4 responses parallel the TSH responses in this condition. But, the normal sized T_3 responses in half of the patients is astonishing and puzzling in view of the virtually absent TSH responses. Calculation of the thyroidal multiplication factor bB, though subject to substantial error and therefore only indicative of the order of magnitude, suggests that in the subgroup of "responders", but also in some of the "non-responders", there must be an extreme sensitivity of the thyroid to stimulation.

In chapter 5 evidence will be presented that TRH is not the direct stimulator responsible for the T_3 response. The most plausible explanation therefore is to assume a minor, but hardly measurable,

secretion of TSH resulting from the prolonged TRH stimulus. A similar immunologically unmeasurable TSH response could be detected in TSH deficiency, by observation of a change in the release rate of thyroidal iodine (101).

When we accept the response to be due to TSH action, the question arises what part of a nodular thyroid does react: the "autonomous" part or the (suppressed) normal part? As it has been observed that single hot nodules are more sensitive to TSH, in vivo (192) as well as in vitro (193), than the suppressed normal tissue, it must have been the nodules that reacted in the cases with a single hot nodule. This might apply to the multinodular cases as well.

In contrast, thyroid tissue of Graves' diseased glands appears to respond to TSH in vitro with normal sensitivity, concerning the adenylcyclase-cAMP-protein kinase system (200) and the phospholipid synthesis (199). But, the fractional response to TSH of the incorporation of radioactive iodine was found to be about twice the response in normal tissue (199), though the iodine concentration must have been much higher in the diseased tissue than in the normal tissue because of the iodine medication before surgery. This might indicate that the thyroid tissue in Graves' disease is oversensitive to TSH as regards hormone synthesis and this might be the explanation for the observed T_3 response in this study.

Whether the hypersensitivity has to be explained by an increase of the number of TSH receptor sites or by a disturbance somewhere along the intracellular route between stimulus signal and ultimate secretion response, can not be answered. But, the demonstration of a T_3 response to TRH in hot nodules will surely be accepted by some (192,193), as evidence for the theory that those nodules originate from and remain active by focal hypersensitivity to TSH.

4.8.7 *Conclusions*

Prolonged infusion of TRH enabled us to study simultaneously the reactions of both pituitary and thyroid to stimulation. In this way it could be shown that two of the main parts of the thyroid hormone control system, the pituitary and the thyroid, are able to compensate a

failure of the other part by adaption. This phenomenon can be observed easily in extreme situations, but could be shown in normals as well. In so-called preclinical primary hypothyroidism the functional loss of thyroidal function is already massive, though euthyroidism persists thanks to an adaptive increase in thyrotropic cell number. Further progression of thyroid failure may ultimately lead to clinical hypothyroidism. The results in patients in which the rate of loss of functional thyroid tissue was high, indicate that the adaption of the pituitary is a time consuming process. Therefore only a slowly progressing decrease of thyroid function can be compensated by the pituitary adequately. In other patients evidence was obtained that a decrease in pituitary thyrotropic function can be compensated by thyroidal hypersensitivity. Also this kind of adaption is limited: hypophysectomy leads to hypothyroidism.

Failure of the third main part of the control system, the hypothalamus, can not be compensated sufficiently. The results in two patients with idiopathic hypothalamic hypothyroidism indicate that the pituitary as well as the thyroid remain basically normal. Yet, as a result of TRH deficiency, their responses to stimulation show some peculiarities. The TSH store in the pituitary appears to be limited, as shown by the exhaustion upon prolonged stimulation, whereas the thyroid seems to be in an inactivated state and therefore needs a lag phase to get into full action.

Surgery in the region of hypothalamus and pituitary may result in a condition similar to idiopathic hypothalamic hypothyroidism. This does not mean that no TRH is produced and/or secreted; it may simply indicate that the portal vessel system of the pituitary stalk has been disrupted or severely damaged during operation, thereby limiting the amount of TRH to reach the thyrotrophs.

Finally, the observed evident T_3 responses in half of the patients with thyroid autonomy are highly interesting. Certainly, these unexpected findings suggest a remarkable hypersensitivity of the thyroid to TSH in these cases. Though the cause for such a hypersensitivity can not be given by this investigation, the observation might be interpreted as further evidence for pituitary-

dependency of hot nodules and for a disproportionality in response of thyroid hormone production, compared to other intra-thyroidal processes stimulated by TSH, in Graves' disease.

The conclusion that the pituitary as well as the thyroid can adapt to a gradual decrease of the total loop gain, harbors some extremely important implications regarding the diagnostic use of serum TSH-determinations. We have seen in chapter 2 that the basal serum TSH level is, apart from its elimination rate, the product of the number of thyrotrophs (A) and the severity of hypothyroidism (error signal = N-E). This implicates that the basal TSH level does not inform us of the severity of hypothyroidism, as long as no information is obtained on the functional capacity of the pituitary. As the TSH-response to exogenous TRH (injection or infusion) only depends on A (the number of thyrotrophs) as long as the same TRH stimulus is given, this response does neither indicate the severity of hypothyroidism. Only the ratio of both parameters is indicative of the degree of deviation from the euthyroidal state:

Q = (basal TSH/TSH response) = (N-E)(constant factor, depending on TRH dose)

However, we do not know the values of N (the TRH stimulus in the basal state) and E (the hormone effect). Yet, the hormone effect is similar to FT_3F, when the individual variation of the target organ sensitivity (C, see chapter 2) is small. Therefore there will be a strongly negative relation between Q and the FT_3F. Because of the individual variation of N and C, there will be considerable deviation from the theoretical straight line in case N and C were constant.

The relation calculated for the normals and the patients with primary hypothyroidism, is graphically depicted in figure 4-3. The TSH response at 2 hours of TRH infusion was used in these calculations to prevent a profound influence of TSH exhaustion.

It is evident that the value of Q is low in normals and indistinguishable from that of the preclinical hypothyroid patients (in patient Lou., in whom no FT_3F could be measured, Q = 0.08). Similar values of Q are found in hypothalamic hypothyroidism (also indicated in fig. 4-3). This was expected, as in this condition the error signal (N-E) is of normal size. Because N is low in this condition, we

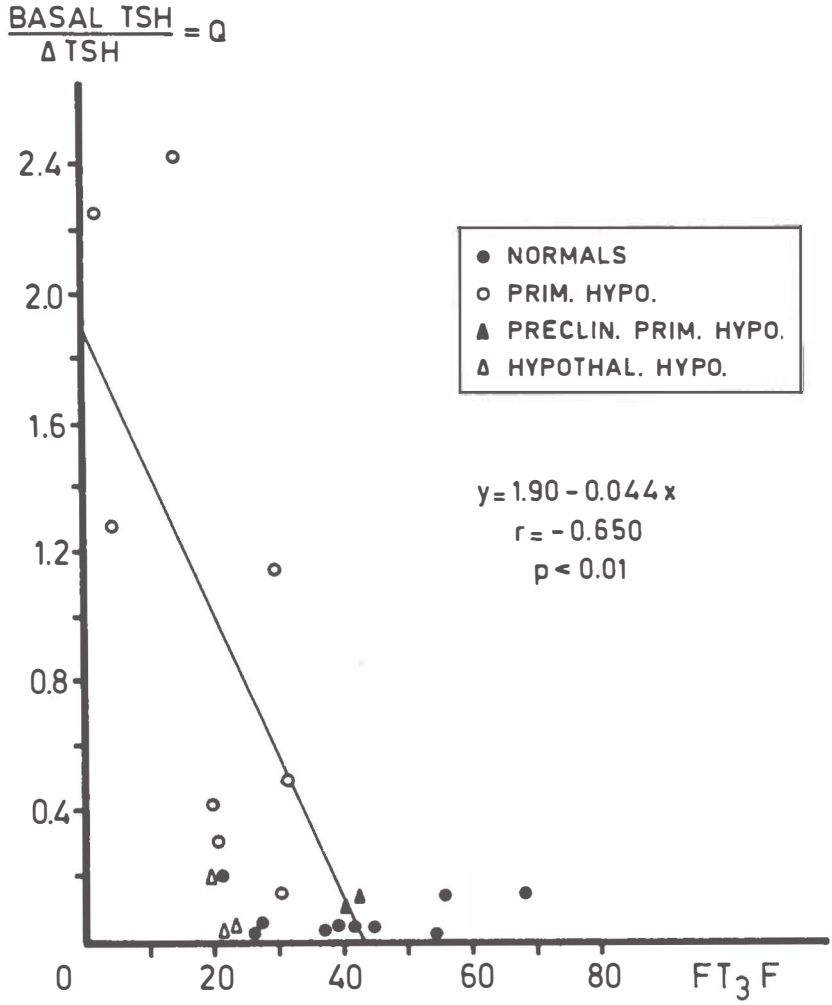


Figure 4-3. The relation between the ratio of basal serum TSH and the TSH response to TRH, and FT_3F. The values of hypothalamic hypothyroid patients were excluded from the calculation of the regression line.

find the hypothalamic patients at the left side of the normals in fig. 4-3. Evident clinical hypothyroidism is indicated by an increase of Q. It is easily seen that the ratio Q is an indicator of the severity of hypothyroidism, in contrast to the basal TSH value or the TSH response.

If we take as upper normal limit of Q 0.20, we can calculate the upper normal limit of the error signal (N-E). We find the upper normal limit of (N-E) to be about 10% of N, which means that in stable euthyroidism the hormone effect has some value between 90 and 100% of the value "prescribed" by the hypothalamus. Such a small range sharply contrast with the observed normal range of FT_3F (or in general of T_3), which indicates that there is substantial variation in either the individual target organ sensitivity to T_3 or the individual hypothalamic secretion of TRH (or of both).

Summarized, the results of the infusion studies have two implications, important for clinical practice. The first one concerns the interpretation of serum TSH levels. The magnitude of the basal TSH level does not represent the severity of primary hypothyroidism. Only the ratio of the basal TSH level and the TSH response to TRH is indicative of the severity of the hypothyroidism.

The second implication concerns the normal range of the serum T_3 level. The range within which the serum T_3 level fluctuates in an individual is only a small part of the observed normal range of the whole population. Therefore the finding of a "normal" value of the serum total or free T_3 level does not exclude a malfunction of one of the three main parts of the control system: hypothalamus, pituitary and thyroid.

Chapter 5

TRH INJECTION STUDIES

5.1.1 *The aim of TRH injection in patients with an autonomous thyroid function*

The response of T_3 during TRH infusion in patients with an autonomous thyroid function, reported in chapter 4, raised the question whether it could be the result of direct TRH-action. As an evident T_3 response was found in only about half of the patients, it is highly unlikely that it resulted from a TRH-mediated enhancement of peripheral T_3-production or from a TRH-mediated redistribution of T_3 over its various compartments. Also the magnitude of the response argues against such an explanation. Therefore, if there was any direct TRH-action involved, this could only have been a stimulus to the thyroid. Such a direct action, involving some sort of hormone-receptor interaction, should be measurable after a high-dosed, but short-lived, stimulus, thereby eliminating as far as possible any time consuming indirect action. Accordingly, a 500 μg bolus injection of TRH was administered to a group of patients with an autonomous thyroid function, in order to study whether any T_3 response comparable to that found in the group of "responders" during infusion (>35 ng/100 ml in 2 hours) could be observed.

5.1.2 *The results of TRH injection in thyroid autonomy*

In six patients with a diagnosed autonomy of the thyroid a TRH-test was performed, using a bolus injection of 500 μg. Data on these patients, as well as the measured hormone parameters, are given in table H of the appendix. Apart from the virtually absent TSH-response, no T_3 difference exceeding 25 ng/100 ml was observed in any patient within a 2-hours period. Besides that, in the group no differences could be found between the T_3 levels at the selected intervals. Therefore no evidence could be obtained of a T_3 response comparable to that found in the "responders" during infusion (at

least 35 ng/100 ml, average 60 ng/100 ml in 2 hours). The basal values of T_4, T_3, TSH, RU, FT_4F, FT_3F and FT_3F/FT_4F in the injection group differed neither from those in the group of "responders" nor from those in the group of "non-responders" of the infusion study.

Though the number of patients studied is small, the results of this investigation do not provide any evidence of a direct stimulatory action of TRH on the autonomously functioning thyroid. Therefore, as reasoned before, the most plausible explanation for the observed T_3-response during prolonged TRH infusion is, that the response results from minor amounts of TSH secreted upon this challenge.

5.2.1 *The aim of TRH injections in normals*

The rate of T_3 secretion of about 8 μg/d per μU/ml of endogenous TSH in normals, reported in chapter 4, indicates that the amount of T_3 secreted during a 2-hours period after TRH injection easily exceeds the amount of T_3 normally secreted in 24 hours. This sudden rise of the T_3 pool will result in an increase of the hormone effect which will be sustained for some period by the enhanced rate of peripheral T_3 production due to the concomitantly increased T_4 pool. Because of the increase of hormone effect the secretion of TSH will stop, followed by a stop of thyroid hormone secretion. Therefore the thyroid hormone levels will gradually decrease and may drop below the original levels before the hormone effect has reached its original value. At that moment a repeated TRH-test should still reveal a reduced TSH response. The results of such a repeated TRH-test would be conflicting at first view: reduced hormone levels in combination with a reduced TSH response. Apart from the implications for investigations involving repeated TRH-tests, the results of such a study certainly will add to the general knowledge on hormonal control systems.

Accordingly, two TRH-tests were performed in a group of young normal males. The second test was started exactly 48 hours after the

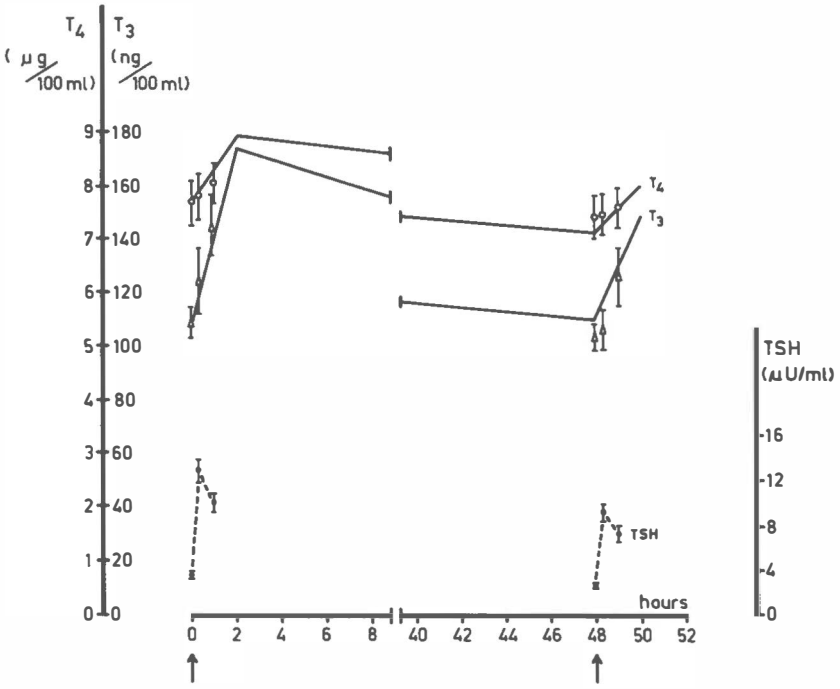


Figure 5-1. Changes in normals during two TRH-tests, 48 hours apart. The means (± sem) are depicted, the solid lines represent the model-predicted changes, the arrows indicate the injection of 200 µg TRH.

first one to exclude any influence of daily rhythm. The injected dose of TRH was chosen at 200 µg, well below the maximal-response-dose of 400 µg.

5.2.2 *The results of the tests in normals*

The measured parameters of repeated tests in 12 normal males are given in table I of the appendix. The mean values (± 1 SEM) of the serum levels of T_4, T_3 and TSH are depicted in fig. 5-1. The probability of the differences within and between the tests are given in table 5-1.

In both tests the TSH response provoked a readily measurable mean T_3 response, but the TSH stimulus is too short-lived and the

TABLE 5-1. Probability values of the changes in normals during two TRH-tests, 48 hours apart. Probability values of the differences between the tests are given in the middle row. The mean values of the parameters are noted in italics for convenience of the reader.

		first test				second test		
	min.	0	20	60		0	20	60
TSH	0	*3.57*			<0.01	*2.68*		
	20	<0.01	*12.91*			<0.01	*9.26*	
	60	<0.01	<0.01	*10.01*		<0.01	<0.01	*7.17*
T_4	0	*7.65*				*7.39*		
	20		*7.81*				*7.45*	
	60			*8.04*				*7.58*
T_3	0	*108.3*				*103.3*		
	20		*124.6*				*107.1*	
	60	<0.01	<0.02	*145.0*		<0.02	<0.01	*126.3*
RU	0	*31.69*				*30.77*		
	20		*31.77*			<0.01	*31.93*	
	60			*31.69*		<0.01		*32.18*
FT_4F	0	*2.99*			<0.05	*2.77*		
	20		*3.05*			<0.05	*2.92*	
	60			*3.14*		<0.05		*3.02*
FT_3F	0	*31.9*				*31.7*		
	20		*39.4*				*33.9*	
	60	<0.01	<0.01	*45.9*		<0.01	<0.01	*40.6*
ΔTSH_{20-0}			*9.34*		<0.01		*6.58*	

interval studied is too short to obtain an evident T_4 response. Interestingly enough, the free T_4 level at the start of the second test was below that of the first one, whereas the free T_3 levels did not differ. Yet, the TSH secretion was inhibited more in the second test than in the first one, as indicated by both the lower basal TSH level and the lower TSH response.

5.2.3 *Substitution of the results in the model*

As before, we omit variations in distribution volumes by taking $V_4 = 11$ L, $V_3f = 22$ L and $V_3s = 18$ L. When we take the total T_4 pool at the start of the second test to be 92.6% of that at the start of the first

one, as indicated by the FT_4F values, one can calculate the enhanced, apparently constant, secretion rate of T_4 occuring during an interval after TRH-injection. This interval appears to be about 2 hours (107). Taking this value, we find a T_4-secretion rate of 1777 μg/d and accordingly the total T_4-pool follows

$TT_4 = -13967 \exp(-0.12t) + 14808$ during the first 2 hours and

$TT_4 = 981 \exp(-0.12t)$ afterwards.

When we take $k_3 = 0.03$, $b = 0.10$ and a constant flow from the slow T_3 pool to the fast one equal to that in the basal situation, we find:

$TT_3f = -108.2 \exp(-1.2t) - 388.0 \exp(-0.12t) + 520.0$ and

$TT_3f = 9.1 \exp(-1.2t) + 27.3 \exp(-0.12t) + 1.7$ respectively.

These curves for T_4 and T_3 are visualized in fig. 5-1, showing a good fit to the observed values.

Subsequent to the rising hormone levels, the hormone effect changes as well:

$E = C[2260.6 \exp(-0.2t) + 108.2 \exp(-1.2t) - 4849.6 \exp(-0.12t) + 2599.9]$ and

$E = C[-220.7 \exp(-0.2t) - 9.1 \exp(-1.2t) + 340.8 \exp(-0.12t) + 8.5]$

respectively. From this it can easily be calculated that the hormone effect at the start of the second test is 107.4% of the effect at the start of the first test, which is about as high as the maximum of 107.6% reached after 40 hours.

As shown in chapter 2, the effect must be about 125% of normal for a complete blockade of the TSH response in a TRH-test. This means that in this study a reduction of about 30% of the TSH response of the second test is predicted by the model. The same response reduction is implicated for the responses of T_3 and T_4. The observed responses of TSH, T_4 and T_3 in the second test were 72, 49 and 63% respectively of those in the first test.

It should be noted, that no attempt was made to become informed of the length of the interval of enhanced thyroid hormone secretion after TRH by additional blood sampling, as this was not of primary interest and not crucial for the investigation. This decision could be made after calculations in advance. These had learnt, that the predicted values of T_3 and E at 48 hours were hardly influenced by the

length of the assumed hormone secretion interval, if chosen within reasonable limits, when the actually measured T_4 levels at 0 and 48 hours were used in the calculations. This supposition can be verified now. When we assume an apparent period of constant secretion of three hours in stead of two, we calculate lower apparent secretion rates of T_4 and T_3 of 1175 and 117.5 μg/d respectively. Nevertheless, the predicted values of T_3 and E at 48 hours remain the same.

5.2.4 *Conclusions*

The sudden change in secretion rate of thyroid hormones during a TRH-test in normals can be concluded to be high enough to result in a subsequent increase in hormone effect. Though clinically insignificant, such an increase can be detected easily with a sensitive test. The changes of such a sensitive test, the TRH test, are fully predictable by the model described in chapter 2.

Apart from the theoretical aspects of interest for the understanding of the functioning of hormonal control systems, these results constitute a warning against investigations based on repeated tests or on several related tests performed within a short interval. As nowadays the tests become more and more sensitive, subsequent tests can influence each other substantially, as shown here for TRH tests. The existence of such an influence can often not be detected by basal hormone measurements, because of the differences in kinetics involved. On the contrary, the measurements may seem to be in conflict at first glance as is the case in this study: the second TSH response is reduced though the free T_4 level is lower than in the first test.

Finally, the fact that the changes found in this study could be sufficiently predicted by the model, again underscores its proper function.

Chapter 6

AUTOIMMUNE ANTIBODIES AGAINST T_3

6.1 INTRODUCTION

In the first chapter we have seen that the immune system is involved in several thyroid abnormalities. Circulating antibodies against thyroidal components are not uncommon even in a "normal" population (179,181). The antibodies can be subdivided depending on the method of detection. By immunofluorescence for instance, they can be divided in cytoplasm - and colloid antibodies.

As thyroglobulin is the most abundant constituent of the colloid, most of the colloid antibodies turn out to be thyroglobulin antibodies. Thyroglobulin antibodies are mostly detected by passive haemagglutination using tanned red cells. Sometimes a colloid positive serum shows a negative tanned red cell test, which indicates that the colloid contains more antigens besides thyroglobulin.

6.1.1 *Thyroglobulin antibodies*

Thyroglobulin is a large glycoprotein with a molecular weight of 660.000. It is strongly immunogenic and has some thirty determinant groups. One of these is the triiodothyronylgroup. As thyroglobulin is present in the blood, though in low amounts (338), a species is normally tolerant for its own thyroglobulin. By injection of thyroglobulin of a different species this tolerance can be broken, resulting in the appearance of cross-reacting antibodies and a clinical picture resembling autoimmune thyroiditis.

Because the T_3 group is an immuno-determinant of thyroglobulin, the first antibodies usable for radioimmunoassay of T_3 were raised in rabbits by means of thyroglobulin injection (339).

Skipping the various theories (135,340) on the maintenance and breaking of tolerance, it is obvious that tolerance can be broken without contact with a heterologous immunogen: autoimmune disease. Circulating thyroglobulin autoimmune antibodies may

consist of a number of subpopulations, each reacting with its own determinant group on thyroglobulin. Logically, one of these subpopulations will be directed to the T_3 determinant and hence able to bind the free hormone T_3 as well. Indeed, significant immunoglobulin binding of T_3 could be detected in the serum of patients with thyroid disease, having hyperthyroidism or primary hypothyroidism (341).

In general, the subpopulation of thyroglobulin antibodies directed against T_3 is inconspicuous (341). However, we encountered two persons with large amounts of this subpopulation of antibodies. Several features of these antibodies were studied in them, as reported below. During this study only one other report on a similar case was published (342).

6.2.1 *Theoretical aspects of antigen binding to its antibody*

Two parameters of an antibody are of special interest: firstly its serum concentration, normally expressed as the total binding capacity for its antigen, and secondly the binding affinity for its antigen, normally expressed as the equilibrium dissociation constant. Both parameters can be determined by tracer binding studies in samples enriched with different amounts of antigen. A third parameter, the antibody specificity, can be judged from the capability of antigen-related compounds to displace tracer antigen.

In the case of circulating anti-thyroglobulin antibodies directed against T_3 we encounter a peculiarity. Because T_3 is bound by them, the free T_3 level will drop. This drop is noticed by the hypothalamus-pituitary control system, resulting in activation of the thyroid, until the original free T_3 level is reached again. This means that, in case of high affinity antibodies, almost all antibody binding sites are occupied. As T_3 is small enough to occupy both binding sites of an antibody independently, we encounter in a serum sample this situation:

$$Ab + T_3 \underset{k_1}{\rightleftharpoons} AbT_3 + T_3 \underset{k_2}{\rightleftharpoons} Ab(T_3)_2$$

As $[Ab] = k_1 [AbT_3]/[T_3]$ and the control system takes care that $[T_3] \gg k_1$, it follows that $[Ab] \ll [AbT_3]$. This means that it seems as if all unoccupied binding sites are located on AbT_3. Upon addition of exogenous T_3 we notice only changes of the second equilibrium: $AbT_3 + T_3 \rightleftarrows Ab(T_3)_2$. Expressed in terms of total capacity (Cap), unoccupied binding sites (UBS), free (F) and bound (B) hormone: $UBS + F \rightleftarrows B$.
Therefore $B/F = UBS/k = (Cap - B)/k$. When tracer hormone is added and we measure the fractions of bound and free radioactivity (B^* and F^* respectively) at equilibrium, we find $B^*/F^* = (Cap - B)/k$. A plot of B^*/F^* against B (a Scatchard plot) yields a straight line with a slope of $-1/k$, intercepting the B-axis at $B = Cap$. Such a Scatchard plot is normally used to disclose the values of Cap and k.

However, when a high amount of thyroglobulin is present in the serum, it will, with its T_3-determinant, compete with the free T_3 for antibody binding sites. But thyroglobulin is so large, that probably only one binding site per antibody molecule can be occupied, thereby "hiding" the other one. (In reality the second binding site can become occupied, but, because of a strongly negative allosteric effect, only at very high levels of thyroglobulin.) So this seems to be the situation:

$$\begin{array}{ccccc} Ab + T_3 & \rightleftarrows & AbT_3 + T_3 & \rightleftarrows & Ab(T_3)_2 \\ + & & k_1 & & k_2 \\ Tg & & & & \\ \updownarrow k_3 & & & & \\ AbTg & & & & \end{array}$$

We know that $[Ab] = k_1 . [AbT_3]/[T_3]$ and $[AbTg] = [Ab] [Tg]/k_3$.
Therefore $[Ab] + [AbTg] = [AbT_3] . (k_3 + [Tg]) . k_1/k_3 . [T_3]$.
In terms of sites not occupied by T_3 it looks as if all sites not occupied by T_3 are located on Ab-Tg, as
$[Ab] \ll [AbT_3]$ because $k_1 \ll [T_3]$ and
$[AbT_3] \ll [AbTg]$ as long as $k_3 . [T_3] \ll k_1 . [Tg]$.
Upon addition of exogenous T_3, it looks as if when one T_3 molecule replaces one Tg molecule, a second T_3 molecule is automatically bound as well: $AbTg + 2\ T_3 \rightleftarrows Ab(T_3)_2 + Tg$.
This resembles an allosteric mechanism with positive homotropic

interaction; indeed, the apparent value of k_1 (=kap) can become very high: $kap = k_1 . (k_3 + [Tg])/k_3$.
In terms of total capacity, unoccupied sites, free and bound hormone, we find UBS + 2 F $\rightleftarrows$ B, with $k = kap.k_2$.
So $B/F^2 = (Cap - B)/k$.

We know that $B^* = B/(B+F) = B/T$ and $F^* = F/T$, and thus $B/F^2 = B^*/(F^*)^2T = (Cap - B)/k$. A plot of $B^*/(F^*)^2T$ against B results in a curve intercepting the B-axis at B = Cap. The slope -1/k will depend on the amount of Tg present: the curve flattens with rising Tg levels. When $[Tg] \gg [AbTg]$ already in the sample not enriched with T_3, k can be considered constant and therefore the curve turns out to be a straight line.

6.2.2 *Determination of Cap and k of T_3 antibodies*

Tracer binding studies were performed as described in chapter 3 under "assay of T_3". However, only "blank binding" tubes were run and of each serum a series of samples was assayed with 0.05 ml buffer replaced by 0.05 ml of T_3 solution in buffer of known concentration (0 to 10 μg/100 ml). Also the "blank binding" of a sample of a normal serum pool (with a negative tanned red cell test) was measured. The blank binding in this normal serum was used to correct the binding in the patient sera. When the fraction of radioactivity apparently bound by the normal serum pool is Bn, the corrected B^* in the patient serum was calculated as
B^* = supernatant counts - Bn(total counts)/(1-Bn)(total counts).
Bn was low, amounting 4 to 6%.

To avoid incomplete mixing of the tracer, the incubation was prolonged to seven days.

In order to calculate $B^*/(F^*)^2T$, we had to know T, which means that we had to know the total amount of T_3 already present in the patient serum before any T_3 addition. Therefore aliquots of patients sera were vigorously treated with 10 volumes of 96% ethanol after addition of one drop of 6N HCl to deproteinize the sample and to liberate T_3. Aliquots of the supernatant were dried in tubes under a stream of nitrogen. The tubes were further treated in the T_3 assay as

zero standard tubes. The amount of T_3 was afterwards read on the standard curve.

When the amount of T_3 present in the patient serum was found to be R ng/100 ml and the concentration of T_3 in the added solution S, then $T = Q + R + S$. The tracerconcentration Q in this equation was measured to be 50 ng/100 ml (see chapter 3). After plotting $B^*/(F^*)^2T$ against $B = B^*.T$ we found Cap. As Cap was expressed in terms of the undiluted serum, the slope of the line had to be corrected for the dilution factor of 13 of the serum in the assay vessel to disclose the real value of k.

6.2.3 *Determination of cross-reaction of the antibodies with T_4*

To study any cross-reaction of the antibodies with T_4, tracer displacement studies were performed essentially as described above, by addition of known amounts of T_4 instead of T_3. The highest T_4 concentration used was 10 μg/100 ml.

6.3 STUDIES IN TWO PATIENTS

6.3.1 *Patient Lou*

Patient Lou, a 16 year old boy, was referred to the endocrinology department because of the development in the past six months of a small painless goiter. His chief complaint concerned his bad results at school, that had been excellent before. Other complaints suggestive for hypothyroidism were only vague. Blood samples were drawn for assay of T_4, resin uptake, TSH, PBI and thyroid antibodies. Two days afterwards a TRH infusion study was performed of which the samples were stored. As the thyroid function parameters determined at admission (see table 6-1) were not alarming and as he was clinically euthyroid, it was decided to see the patient a second time after 2 months before starting any medication. As by this time the data of the TRH infusion study (see table E of the appendix) were known, except for the T_3 values, and as the goiter had grown further, it was decided to provide substitution therapy with desiccated thyroid. His school

results improved together with the regression of the goiter. Shortly thereafter the T_3 determinations were performed, indicating the presence of T_3 binding antibodies. A closer look at the thyroid parameters on the day of the decision to treat revealed normal values of T_4 and TSH, but yet it was decided to maintain the substitution therapy, because of the presence of T_3 antibodies. He was seen regularly thereafter in follow up.

TABLE 6-1. Some data of the two patients.

Interval from TRH study	T_4	RU	TSH	PBI	cyto-ab.	coll-ab.	TRC-test	T_3-ab.	Total T_3 ng/dl
patient Lou.									
-2 days	5.5	20.2	11.3	7.2	str.pos.	w.pos.	neg.	pos.	2600
0	5.0	23.1	9.0					pos.	2900
8 weeks	7.0	28.0	3.6					pos.	1200
19 weeks	4.9	27.2	0.5		str.pos.	w.pos.	neg.	pos.	700
28 weeks	5.9	29.9	<0.5					pos.	400
58 weeks	6.7	32.2	<0.5					neg.	270
67 weeks	7.8	36.7	0.6						
101 weeks	4.4				pos.	w.pos.	neg.		
106 weeks	4.8	29.0	1.9						
118 weeks	5.8	28.2	4.5	3.0	pos.	w.pos.	neg.	neg.	225
145 weeks	4.9	27.6							
patient Juc.									
0 min.	9.4	32.6	<0.5		neg.	neg.	neg.	pos.	750
20 min.			4.3						
60 min.			2.4						

Patient Juc.

The adult female patient Juc. was referred to the department of Psychiatry because of manic depression. There were no complaints suggestive of thyroid pathology, nor any family history in that direction. Physical examination did not reveal any abnormality. The patient entered a program on the study of the role of TRH in manic depression (30), in which several thyroid parameters were determined. Surprisingly, though the routine thyroid parameters were completely normal (table 6-1), including the TRH test and the absence of antibodies to colloid and cytoplasm, T_3 binding antibodies

were detected. Any further follow up was impossible unfortunately, as she never attended the hospital again.

6.3.2 *Results*

Scatchard plots of the tracer binding studies failed to result in straight lines for some of the sera of patient Lou. and for all sera of patient Juc. It was decided to try a plot of $B^*/(F^*)^2T$ against B, for reasons described above. For all sera these plots yielded straight lines, enabling the determination of the capacity and apparent equilibrium constant of the T_3-antibody.

a. *The studies concerning patient Lou.*

In several sera of patient Lou., sampled at different moments, the antibody capacity for T_3, the apparent dissociation equilibrium constant and the total amount of T_3 were determined. At admission, the total T_3 level is very high and equal to or just above the antibody capacity, indicating the high affinity of the antibodies. (The corrected binding percentages are given in table J of the appendix).

TABLE 6-2. Parameters concerning T_3-antibodies in patient Lou., during and after infusion of 1000 µg TRH in 4 hours.

Interval hours	Total T_3 ng/dl	Binding cap. ng/dl	Equil.diss.constant $10^{-20}mol^2/l^2$
0	2900	2650	4.7
2	2900	2650	4.7
4	3350	2700	5.6
6	3450	2750	4.7
8	3600	2800	3.1
24	2350	3000	43.6

The results in the sera sampled during and after TRH infusion reveal the secretion of large amounts of T_3 (table 6-2), caused by the very high levels of TSH (see table E of the appendix). Yet, when the values of bB and TLG are calculated as in chapter 4, we find values of 2.6 μg.ml/μU.d and 743 μg/d respectively. This indicates that the total loop gain of the whole system is quite normal, but that the production of thyroid hormone is hardly high enough to match the high demand for T_3 caused by binding of T_3 to the antibodies and subsequent removal.

The TRH study yields two other interesting observations. Firstly, the apparent value of k markedly rises from values of about 5. 10^{-20} M^2/L^2 to 44. 10^{-20} M^2/L^2 at 24 hours after the infusion start, indicating the secretion of substantial amounts of thyroglobulin or parts of it (fig. 6-1). The level of this material becomes high enough to displace T_3 from the antibody binding sites, resulting in a total T_3 level below the antibody capacity (table 6-2). Secondly, the antibody capacity rises gradually during and after TRH infusion, probably through stimulation of antibody production by the secreted thyroglobulin material.

The results of the antibody capacity determination during follow up (fig. 6-1) indicate a decrease, that already had started before substitution therapy had been given. The decrease is nicely exponential (fig. 6-2), with a half life of about seven weeks. This half life is surely different from the biological half life of about 25 days of igG molecules and probably refers to the biological half life of sensitized B-lymphocytes. After 58 weeks the T_3 antibodies become undetectable and do not show up again (table J of the appendix). During follow up the apparent value of k is higher than in the initial samples. Whether this must be explained by a gradual decrease of affinity of the antibodies produced by the surviving plasma cells, or by acceptance of circulating thyroglobulin is uncertain.

The antibodies to T_3 were specific in the sense that T_4 was unable to displace T_3 (table K of the appendix). Though there was no reason to suggest a change in specificity, a sample at an other moment during follow up was tested, with the same result.

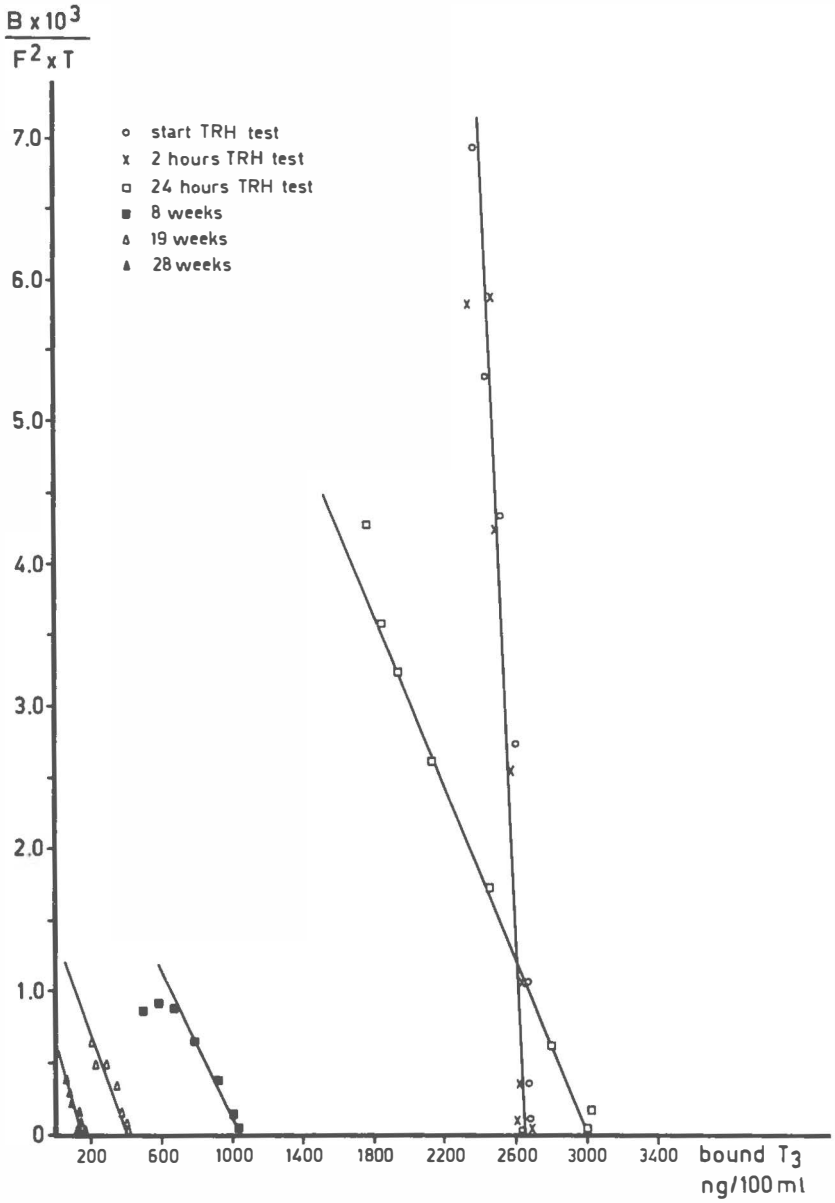


Figure 6-1. Estimation of the T_3 binding capacity and the apparent equilibrium dissociation constant in sera of patient Lou. Samples were taken at the start of the 4 hours' infusion of TRH, two and 24 hours thereafter, and during follow up.

In the face of the disappearing antibodies to T_3, it was of interest to become informed of the titers of thyroid antibodies determined by immunofluorescence and haemagglutination. Though originally tested in different runs, stored samples were reassayed in one single run (see table 6-1) for proper comparison. The anti cytoplasm titer slowly decreased during follow up, in contrast to the anti colloid titer, that did not fall at all. Surprisingly, the tanned red cell test was negative in all samples assayed.

b. *Results concerning patient Juc.*

Antibodies to T_3 were detected in this psychiatric patient. Before and 8 hours after injection of 200 μg TRH the values of total T_3, T_3 binding capacity and apparent k were: 750 ng/100 ml, 570 ng/100 ml, 62×10^{-20} M^2/L^2 and 850 ng/100 ml, 570 ng/ml, 80×10^{-20} M^2/L^2 respectively. As all other thyroid tests were normal, and no antibodies were detectable by immunofluorescence or haemagglutination, we are left with the observation that high affinity T_3 antibodies may circulate in rather high amounts without causing any clinical sign or abnormal routine thyroid test result (see tables 6-1 and L).

6.4 DISCUSSION

From a theoretical point of view the finding of anti-thyroglobulin antibodies binding T_3 is not unexpected. Yet, when present, this subpopulation of antibodies forms normally a minority (341), which might indicate that thyroglobulin has a number of determinants that are much more immunogenic than the T_3-determinant. Therefore it is strange that in both of the patients described here, the tanned red cell was negative. As in the patient with high levels of T_3-antibodies described by others (342) the anti-thyroglobulin titer was barely above the limit of detection, the absence of a high anti-thyroglobulin titer might be a special feature in this kind of auto-immunity.

There are striking differences as well: the apparent dissociation equilibrium constants in our patients are much lower than in the other case, with a value of 2×10^{-9} M/L (342). As this higher value is in the same order of magnitude as that of TBG, it is understandable that in this case not all binding sites were occupied: the reported values of capacity and total T_3 level are 13000 ng/100 ml and 2300 ng/ml (342).

Most remarkable in patient Lou. were the changes in the level of T_3-antibodies. On the one side the level could be raised by the secretion of thyroglobulin through TRH-infusion, on the other side there was a slow exponential decrease of the level, independent of substitution therapy. An interpretation of these facts could be that proliferation of B-lymphocytes, responsible for the T_3 antibody, had already stopped by the time the patient was referred. The set of plasma cells and their precursors, present at that moment, then disappeared with a half life of about 7 weeks. The rise of the antibody level upon TRH stimulation might be explained by a transitory enhancement of the secretion of antibody by the plasma cells present.

The fact that the tanned red cell test was negative, together with the disappearance of the T_3 antibody in contrast with the stable titer of colloid antibodies, indicates that the colloidal antigen recognized by the colloid antibodies was different from thyroglobulin. That substitution therapy did not change the kinetics concerning the levels of both the T_3 antibodies and the colloid antibodies favours the idea that the state of activity of the thyroid is of little importance in the mechanisms involved in the maintenance of production of thyroid antibodies. Why and how the fate of antibody production to thyroidal components can be so different from antigen to antigen is intriguing, but can not be answered by this study.

Just as is the case with "normal" thyroid antibodies, T_3-antibodies do not necessarily cause clinically evident thyroid dysfunction. Patient Juc. is a perfect example in this respect.

Both case histories show that one should at least be alert when the measured T_3 levels do not match the clinical picture, or, even better, that one should check blank binding in each sample for T_3 assay, in order to detect minimal levels of T_3 binding antibodies.

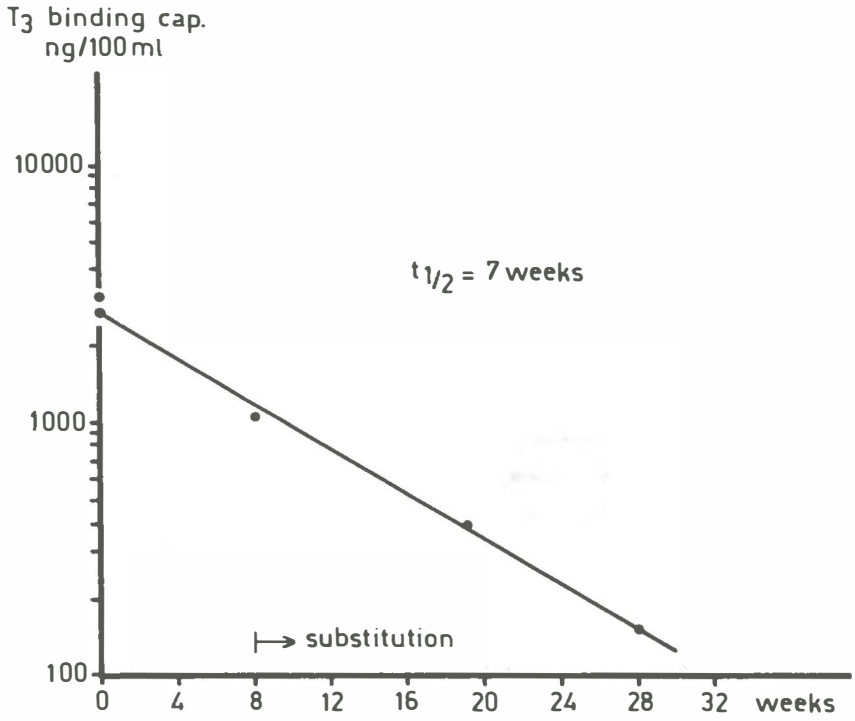


Figure 6-2. The exponential fall of the binding capacity for T_3 during follow up of patient Lou. Medication with desiccated thyroid was started at 8 weeks from admission.

SAMENVATTING

In hoofdstuk 1 wordt een overzicht gegeven van hetgeen bekend is over de schildklierfunctie. Het hormonale controlesysteem dat de productie van de schildklierhormonen thyroxine (T_4) en trijodothyronine (T_3) reguleert, bestaat uit een aantal componenten, waarvan de hypothalamus, de hypophyse en de schildklier zelf veruit de belangrijkste zijn. Het in de hypothalamus gesynthetiseerde thyroliberine (TRH) zet de hypophyse aan tot productie en secretie van het schildklier-stimulerend-hormoon (TSH). Dit hormoon activeert op zijn beurt in de schildklier een aantal processen, die culmineren in de productie en afgifte van de jodiumhoudende hormonen T_4 en T_3. Via de bloedbaan bereiken deze de organen en weefsels waar ze hun effect uitoefenen. Een groot aantal energie-vergende processen wordt door dit effect beinvloed. Vandaar dat het noodzakelijk is de schildklierhormoonproductie zeer nauwkeurig te reguleren. Dit wordt bereikt via één van de effecten van schildklierhormoon: de remming van de afgifte door de hypophyse van TSH. Op deze wijze is een soort thermostaat voor de schildklierfunctie gecreëerd, waarbij de hoeveelheid afgegeven TRH de afstelling weergeeft. Het schildklierhormoon effect bepaalt of de thermostaat aanslaat (TSH-afgifte) om de afgifte van schildklierhormoon te stimuleren, totdat het schildklierhormoon effect weer zoveel gestegen is, dat de thermostaat afslaat.

Omdat hypothalamus, hypophyse en schildklier zo belangrijk zijn in het totale controlesysteem, zijn abnormaliteiten in de schildklierfunctie dan ook vrijwel steeds terug te voeren op afwijkingen in één van deze drie. Echter ook andere, al dan niet physiologische, processen spelen een rol in de regulering van de schildklierfunctie, en zijn daarom besproken.

Eén daarvan vervult een niet onaanzienlijke rol. Het is gebleken dat T_4 zelf geen of een slechts zeer miniem effect sorteert, en dat het buiten de schildklier, vooral in de lever, moet worden omgezet in T_3 om wel effect te hebben. Omdat normaal 70% van de totale T_3-productie via deze omzetting tot stand komt, kan beinvloeding van de

omzetting van grote importantie zijn en zelfs de functie van veiligheidsklep krijgen in situaties waarbij zoveel mogelijk energie moet worden bespaard. Dit blijkt ook het geval te zijn. In omstandigheden van acute stress, ondervoeding en koolhydraattekort komen bijnierschorshormonen vrij, die in staat zijn het enzyme dat de omzetting van T_4 en T_3 verzorgt, volledig zijn activiteit te ontnemen. Aangezien ze gelijktijdig ook de TRH-afgifte blokkeren, wordt aldus simultaan de productie van T_3 in en buiten de schildklier stopgezet. Omdat een der afbraakproducten van T_4, het reversed T_3 (rT_3), door hetzelfde enzyme dat T_4 in T_3 omzet verder wordt gemetaboliseerd, is het duidelijk dat in de situaties dat de T_3-productie wordt stopgezet, tegelijkertijd de rT_3-concentratie stijgt. Het rT_3 heeft echter geen biologische werking.

In hoofdstuk 2 is een beschrijving gegeven van een eenvoudig kinetisch model van de schildklierhormoonregulatie. Een belangrijk verschil met andere, ingewikkelde, modellen vormt het schildklierhormoon effect, beschreven als een aparte biochemische "stof" met een lange biologische halfwaardetijd. Met behulp van dit model blijken de waarnemingen van andere auteurs uitstekend verklaard te kunnen worden. De later beschreven resultaten van het onderzoek zijn eveneens aan de hand van dit model bewerkt.

Hoofdstuk 3 omvat de gegevens betreffende de uitgevoerde testprocedures, de personen die deze ondergingen en de laboratoriumbepalingen die bij het onderzoek werden toegepast.

De resultaten van de infusie van 1000 μg TRH in vier uur bij verschillende categorieën personen zijn beschreven in hoofdstuk 4. Het blijkt, dat hypophyse en schildklier elkaars falen door aanpassing kunnen compenseren. Een verminderde functie van de schildklier kan gedurende lange tijd dusdanig gecompenseerd worden door vergroting van het TSH-producerende deel van de hypophyse, dat klinisch euthyreoidie blijft bestaan en alleen biochemisch de zgn. preklinische hypothyreoidie aantoonbaar is. Pas wanneer de functie van de schildklier terugloopt tot minder dan een tiende van normaal, of wanneer het functieverlies acuut door b.v. medisch ingrijpen

ontstaat, schiet de aanpassing van de hypophyse tekort en ontstaat het klinisch beeld van primaire hypothyreoidie.

Omgekeerd kan ook de afname van de capaciteit van de hypophyse, door b.v. tumorgroei, gedeeltelijk gecompenseerd worden door de schildklier. Deze aanpassing geschiedt door vergroting van de gevoeligheid van de schildklier voor het TSH, tot waarden vijf maal hoger dan normaal. Een groot acuut capaciteitsverlies leidt echter tot secundaire hypothyreoidie.

Door genoemde aanpassingen kan het regulatiesysteem waarborgen dat het uiteindelijk schildklierhormoon effect bij klinische euthyreoidie nooit meer dan tien procent afwijkt van de door de hypothalamus, via de TRH-afgifte, "voorgeschreven" optimale waarde. Deze waarneming leidt tot twee belangrijke gevolgtrekkingen. De marge van tien procent in hormooneffect is vele malen kleiner dan de waar te nemen "normal ranges" van de serumgehaltes van T_4 en T_3 in een populatie, hetgeen betekent dat er grote individuele verschillen in gevoeligheid voor schildklierhormoon bestaan en dat meting van T_4 en/of T_3 niet zonder meer voldoende is voor een correcte waardering van de schildklierfunctie. Verder kan de capaciteit van de hypophyse door al dan niet opgetreden aanpassing zoveel verschillen, dat noch de hoogte van de basale TSH-spiegel, noch de response daarvan op exogeen toegediend TRH voldoende informatie omtrent de schildklierfunctie kan leveren. Alleen de verhouding tussen deze grootheden geeft inzicht in de ernst van de afwijking.

Een onvoldoend functioneren van de hypothalamus kan niet gecompenseerd worden en leidt tot zgn. hypothalamische hypothyreoidie. In zijn pure vorm blijken hypophyse en schildklier normaal reactief te blijven en levert een TRH-test normale waarden op. Vaak bestaat echter een mengvorm van hypothalamische en secundaire hypothyreoidie, met name bij suprasellaire tumoren en na chirurgie in het betrokken gebied.

Bij een groep patienten met autonome schildklierfunctie bleef de TSH-response tijdens TRH-infusie uit, zoals te verwachten. Slechts bij één, klinisch euthyreoiede, patient werden vrijwel normale

reacties van hypophyse en schildklier waargenomen, hetgeen erop wijst dat de autonome hormoonproductie de behoefte niet te boven ging. Opvallend was, dat bij ongeveer de helft van deze groep patienten een T_3-response werd waargenomen, in grootte vergelijkbaar met die bij controlepersonen. Waarschijnlijk werd door TRH-infusie toch een niet-meetbare TSH-response verkregen, waarop door de abnormale schildklier met verhoogde gevoeligheid werd gereageerd. Nodulaire en multinodulaire afwijkingen onderscheidden zich in dezen niet van de homogene vorm.

In hoofdstuk 5 zijn de resultaten vermeld van TRH-injectie. Bij een groep patienten met hyperthyreoidie werd 500 μg TRH ingespoten om te zien of een kortdurende zeer grote TRH-stimulus, net als de langdurige geringe TRH-stimulus door infusie, tot een T_3-response zou leiden. Dit bleek niet het geval te zijn.

Omdat door berekeningen met behulp van het model was gebleken dat het schildklierhormoon effect in belangrijke mate kan achterlopen op veranderingen in schildklierhormoonspiegels, werd nagegaan in hoeverre een TRH-test een volgende TRH-test zou beinvloeden. Berekening vooraf leerde dat maximale beinvloeding te verwachten was na ongeveer twee dagen. Daarom werden bij een groep normale personen twee TRH-testen met 200 μg uitgevoerd met een interval van 48 uur. Hoewel de T_3-spiegel bij aanvang van de tweede test reeds was teruggekeerd tot het oorspronkelijke basale niveau en de T_4-spiegel zelfs tot beneden dit niveau was gedaald, bleek het verhoogde hormooneffect inderdaad aantoonbaar: de aanvangsspiegel van TSH bij de tweede test was gesupprimeerd en de TSH-response was slechts 70% van die in de eerste test. Deze waarneming houdt een waarschuwing in met betrekking tot de toepassing van meerdere uiterst gevoelige testen binnen enkele dagen, wanneer deze elkaar, via klinisch niet waarneembare effecten, zouden kunnen beinvloeden.

Door de individuele verschillen in orgaangevoeligheid voor T_3, door compensatoire adaptie van hypophyse of schildklier, of door voorgaande diagnostische testen kan men aldus geconfronteerd worden met een ogenschijnlijke discrepantie tussen TRH-test en

basale schildklierhormoonspiegels. Echter ook de aanwezigheid van antilichamen gericht tegen T_4 en/of T_3 kan leiden tot een dergelijke discrepantie. In het laatste hoofdstuk worden de studies beschreven, die zijn verricht bij twee patienten, bij wie grote hoeveelheden T_3, gebonden aan circulerende antilichamen, werden aangetroffen. Bij de eerste patient bleek de antilichaamsbindingscapaciteit, onafhankelijk van schildklierhormoonsubstitutie, exponentieel te verdwijnen met een halfwaardetijd van zeven weken. De bovendien aanwezige antilichamen tegen colloid en cytoplasma veranderden echter niet of nauwelijks in titer gedurende de observatieperiode van meer dan een jaar. Bij de tweede patient, die euthyreoied was en geen symptomen had die wezen op schildklierpathologie, waren alle gemeten overige schildklierparameters normaal, inclusief de TRH-test. De resultaten laten zien dat bij bepaling van T_4 en T_3 rekening gehouden dient te worden met de mogelijkheid van circulerende antilichamen hiertegen, teneinde foutieve interpretatie te vermijden. Opvallend was, dat bij beide patienten antilichamen tegen thyreoglobuline niet aantoonbaar waren.

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A P P E N D I X.

In the tables the units of the parameters are: T_4 in µg/dl, T_3 in ng/dl, RU in %, TSH in µU/ml, whereas FT_4F and FT_3F are given in arbitrary units (see chapter 3).

TABLE A. The parameters of normals during and after TRH infusion.

	age/ sex	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Son.	18/M	0	8.3	170	32.9	3.1	3.40	55.9	16.4
		2	8.6	290	38.5	24.0	4.31	111.7	
		4	9.1	285	40.0	29.5	4.79	114.0	
		6	9.3	265	40.1	9.1	4.91	106.3	
		8	8.6	230	38.4	6.5	4.29	88.3	
		24	8.7	190	36.0	4.3	3.99	68.4	
Han.	17/M	0	8.8	200	34.1	4.4	3.77	68.2	18.1
		2	11.3	290	30.7	35.6	4.25	89.0	
		4	12.2	350	32.1	26.0	4.85	112.4	
		6	11.4	285	31.4	7.4	4.41	89.5	
		8	11.9	270	32.1	3.6	4.73	86.7	
		24	11.4	225	30.8	0.5	4.31	69.3	
Verh.	68/F	0	7.1	65	32.1	3.9	2.82	20.9	7.4
		2	7.8	115	31.5	23.2	3.03	36.2	
		4	9.6	150	32.1	24.6	3.82	48.2	
		6	10.1	165	31.5	13.3	3.92	52.0	
		8	10.2	170	32.1	10.5	4.06	54.6	
		24	8.5	70	31.9	4.7	3.35	22.3	
Schel.	43/M	0	8.2	110	33.6	< 0.5	3.45	37.0	10.7
		2	9.2	220	34.6	19.0	4.02	76.1	
		4	10.7	240	37.5	23.5	5.18	108.8	
		6	12.3	260	37.5	6.6	5.95	97.5	
		8	13.4	285	31.2	3.2	5.14	88.9	
		24	11.4	120	32.6	< 0.5	4.62	39.1	
Band.	20/M	0	10.0	150	27.6	1.3	3.31	41.4	12.5
		2	10.9	245	29.2	31.8	3.86	71.4	
		4	12.7	330	31.4	27.1	4.91	103.6	
		6	12.6	310	30.3	6.3	4.76	95.5	
		8	12.5	255	31.4	1.6	4.84	80.1	
		24	13.4	175	30.6	< 0.5	5.02	53.6	
Vd Vl.	39/M	0	7.9	170	31.8	< 0.5	3.10	54.1	17.5
		2	10.1	315	34.3	27.7	4.36	108.0	
		4	10.7	295	35.7	19.0	4.86	105.3	
		6	11.7	260	34.3	7.3	5.05	89.2	
		8	11.0	245	35.6	2.5	4.98	87.2	
		24	10.0	190	33.2	< 0.5	4.15	63.1	
De Jag.	34/F	0	6.7	150	30.0	1.8	2.45	45.0	18.4
		2	7.6	180	28.0	52.0	2.56	50.4	
		4	8.8	380	30.2	50.0	3.25	114.8	
		6	9.1	410	29.3	13.0	3.24	120.1	
		8	8.6	210	29.0	4.6	3.02	60.9	
		24	8.0	110	29.4	0.5	2.86	32.3	

	age/ sex	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Uitd.	20/F	0	11.0	90	29.1	<0.5	3.88	26.2	6.8
		2	12.3	150	29.1	21.6	4.34	43.7	
		4	13.8	260	30.5	19.9	5.15	79.3	
		6	13.7	345	30.5	1.6	5.11	105.2	
		8	14.9	185	34.5	<0.5	6.48	63.8	
		24	13.5	140	34.6	<0.5	5.89	48.4	
Sla.	42/M	0	11.6	130	29.9	0.8	4.23	38.9	9.2
		2	13.3	155	31.1	16.1	5.09	48.2	
		4	13.5	170	34.2	16.6	5.81	58.1	
		6	14.0	-	30.3	3.7	5.18	-	
		8	15.1	190	27.3	2.4	4.93	51.9	
		24	13.2	140	30.4	0.8	4.91	42.6	
Post.	17/F	0	7.3	95	28.6	1.9	2.52	27.2	10.8
		2	8.4	190	32.2	40.0	3.35	61.2	
		4	11.6	240	35.0	38.4	5.14	84.0	
		6	10.8	290	31.4	14.2	4.18	91.1	
		8	11.8	190	29.7	7.8	4.26	56.4	
		24	10.4	95	32.1	2.4	4.13	30.5	
Mean values		0	8.7	133	31.0	1.9	3.29	41.5	12.8
		2	10.0	215	31.9	29.1	3.92	69.6	
		4	11.3	275	33.9	27.5	4.78	92.9	
		6	11.5	288	32.7	8.3	4.67	94.0	
		8	11.8	223	32.1	4.3	4.67	71.9	
		24	10.9	146	32.2	1.5	4.32	47.0	

TABLE B. The parameters during and after TRH infusion in the "non-responders" of the autonomy category.

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Pip.	0	15.8	210	46.0	2.5	10.04	96.6	9.6
	2	14.8	205	46.0	4.0	9.40	94.3	
	4	15.9	220	45.9	2.2	10.07	101.0	
	6	15.1	200	46.8	4.1	9.83	93.6	
	8	14.8	220	42.6	4.4	8.47	93.7	
	24	14.4	195	47.8	4.3	9.65	93.2	
And.	0	15.3	400	44.4	<0.5	9.26	177.6	19.2
	2	15.3	400	47.9	<0.5	10.28	191.6	
	4	14.3	400	47.1	<0.5	9.38	188.4	
	6	15.0	400	46.1	<0.5	9.56	184.4	
	8	13.9	360	45.3	<0.5	8.65	163.1	
	24	14.0	385	44.9	<0.5	8.60	172.9	
Goe.	0	15.3	350	39.8	0.9	8.00	139.3	17.4
	2	15.2	370	39.0	0.9	7.74	144.3	
	4	14.8	315	40.4	1.4	7.89	127.3	
	6	15.8	-	36.1	1.9	7.28	-	
	8	13.9	340	38.9	1.6	7.05	132.3	
	24	15.4	-	39.8	-	8.05	-	

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Dou.	0	19.6	320	49.0	<0.5	13.60	156.8	11.5
	2	18.8	355	45.5	<0.5	11.77	161.5	
	4	19.6	345	46.0	5.1	12.45	158.7	
	6	19.3	360	43.3	3.0	11.29	155.9	
	8	18.7	395	48.7	0.7	12.87	192.4	
	24	-	-	-	-	-	-	
Wie.	0	15.7	70	38.6	0.5	7.89	27.0	3.4
	2	14.1	80	38.8	4.7	7.13	31.0	
	4	15.0	90	37.9	3.6	7.36	34.1	
	6	16.0	90	38.8	4.5	8.09	34.9	
	8	15.4	70	38.7	3.2	7.76	27.1	
	24	15.9	125	38.0	5.2	7.82	47.5	
Jan.	0	15.0	185	41.9	<0.5	8.40	77.5	9.2
	2	14.9	175	40.7	1.8	8.02	71.2	
	4	14.4	195	39.9	1.1	7.55	77.8	
	6	14.2	185	40.0	0.8	7.47	74.0	
	8	15.2	195	38.2	1.4	7.53	74.5	
	24	13.3	165	40.0	1.7	7.00	66.0	
Zui.	0	13.6	245	46.9	1.3	8.88	114.9	12.9
	2	15.1	275	44.4	2.6	9.14	122.1	
	4	13.9	260	46.3	3.2	8.91	120.4	
	6	14.7	250	46.9	2.3	9.59	117.3	
	8	15.4	270	43.7	3.5	9.12	118.0	
	24	14.7	265	46.0	3.5	9.34	121.9	
Smi.	0	11.2	125	34.0	<0.5	4.78	42.5	8.9
	2	10.0	140	35.9	0.7	4.58	50.3	
	4	11.6	145	38.0	<0.5	5.71	55.1	
	6	11.6	140	34.8	<0.5	5.10	48.7	
	8	11.0	150	34.1	0.9	4.72	51.2	
	24	11.6	200	35.1	<0.5	5.16	70.2	
Beu.	0	10.5	150	25.4	1.3	3.15	38.1	12.1
	2	10.1	180	27.8	2.4	3.37	50.0	
	4	9.3	150	26.9	<0.5	2.98	40.4	
	6	10.4	160	24.7	2.9	3.02	39.5	
	8	10.2	150	27.8	<0.5	3.40	41.7	
	24	10.0	170	27.9	<0.5	3.35	47.4	
Lin.	0	8.0	200	30.5	1.4	2.99	61.0	20.4
	2	7.5	165	29.5	0.8	2.69	48.7	
	4	8.0	185	30.3	1.9	2.96	56.1	
	6	8.4	185	31.0	2.8	3.20	57.4	
	8	8.7	-	30.0	2.0	3.18	-	
	24	8.6	175	28.1	1.9	2.91	49.2	
Van M.	0	11.4	640	32.9	<0.5	4.55	210.6	46.3
	2	12.4	670	33.2	0.7	5.14	222.4	
	4	11.9	640	34.4	<0.5	5.16	220.2	
	6	11.2	680	30.5	<0.5	4.18	207.4	
	8	11.6	650	31.1	<0.5	4.44	202.2	
	24	10.0	500	33.1	0.8	4.13	165.5	

TABLE C. The parameters during and after TRH infusion in the "responders" of the autonomy category.

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Mul.	0	15.8	355	40.6	2.5	8.48	144.1	17.0
	2	15.2	410	39.4	2.6	7.84	161.5	
	4	15.7	445	40.5	2.9	8.40	180.2	
	6	15.9	445	39.0	3.4	8.10	173.6	
	8	14.7	375	40.4	4.2	7.84	151.5	
	24	15.5	290	40.8	0.6	8.37	118.3	
Roo.	0	15.1	315	40.2	1.5	8.00	126.6	15.8
	2	15.0	370	42.2	4.1	8.48	156.1	
	4	-	-	-	-	-	-	
	6	15.0	495	40.4	2.1	8.00	200.0	
	8	14.0	465	39.6	3.2	7.27	184.1	
	24	13.9	410	40.4	2.3	7.41	165.6	
Van A.	0	16.0	280	40.7	1.1	8.62	114.0	13.2
	2	14.7	330	41.5	0.9	8.12	137.0	
	4	14.3	410	39.0	0.7	7.28	159.9	
	6	15.2	305	41.8	0.7	8.48	127.5	
	8	14.7	300	42.0	-	8.25	126.0	
	24	15.8	490	40.6	0.8	8.48	198.9	
De W.	0	18.0	310	41.9	1.3	10.07	129.9	12.9
	2	16.3	355	42.0	3.1	9.15	149.1	
	4	18.2	440	44.0	1.7	10.88	193.6	
	6	16.5	350	41.5	3.5	9.12	145.3	
	8	17.5	375	40.0	1.7	9.21	150.0	
	24	17.2	430	46.0	1.7	10.93	197.8	
Nic.	0	13.7	215	44.3	<0.5	8.72	95.2	11.5
	2	12.3	300	42.9	<0.5	7.11	128.7	
	4	14.8	340	44.7	<0.5	9.04	152.0	
	6	14.2	350	42.5	<0.5	8.10	148.8	
	8	14.8	350	41.0	<0.5	8.05	143.5	
	24	14.6	260	42.1	<0.5	8.22	109.5	
Pou.	0	14.9	130	50.2	<0.5	10.70	65.3	6.1
	2	13.9	170	50.1	<0.5	9.96	85.2	
	4	15.2	240	53.2	1.6	11.88	127.7	
	6	14.9	215	48.6	2.4	10.22	104.5	
	8	14.7	175	49.1	1.4	10.23	85.9	
	24	15.6	130	52.4	2.3	11.92	68.1	
Sto.	0	11.0	250	34.0	1.6	4.70	85.0	18.1
	2	10.9	440	33.9	2.3	4.64	149.2	
	4	11.3	460	31.4	1.4	4.37	144.4	
	6	12.1	450	35.3	0.9	5.42	158.9	
	8	11.3	425	36.6	2.0	5.30	155.6	
	24	11.4	370	33.0	<0.5	4.69	122.1	
Bos.	0	13.4	180	35.8	1.9	6.11	64.4	10.5
	2	13.6	250	35.6	1.7	6.16	89.0	
	4	13.6	265	35.2	2.0	6.07	93.3	
	6	13.1	290	33.1	2.4	5.41	96.0	
	8	13.5	260	34.4	3.0	5.85	89.4	
	24	13.8	215	33.4	1.1	5.76	71.8	

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Bro.	0	9.4	135	28.2	2.0	3.19	38.1	11.9
	2	10.8	170	28.6	13.0	3.73	48.6	
	4	11.5	205	28.2	11.4	3.90	57.8	
	6	12.2	200	24.8	3.9	3.55	49.6	
	8	11.6	230	26.8	3.6	3.70	61.6	
	24	11.4	175	26.4	1.3	3.58	46.2	

TABLE D. The parameters during and after TRH infusion of patients in the category of primary hypothyroidism.

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Vd M.	0	4.8	110	27.5	19.5	1.58	30.3	19.2
	2	5.4	155	26.7	150.8	1.72	41.4	
	4	5.9	125	29.1	159.3	2.08	36.4	
	6	5.6	165	29.9	103.1	2.04	49.3	
	8	5.1	145	30.0	48.9	1.87	43.5	
	24	5.5	155	29.2	10.1	1.95	45.3	
Col.	0	4.3	120	26.1	45.1	1.33	31.3	23.5
	2	3.2	175	24.7	135.3	0.93	43.2	
	4	3.1	190	26.8	132.8	0.99	50.9	
	6	3.0	185	26.7	84.4	0.95	49.4	
	8	3.2	130	28.8	44.7	1.11	37.4	
	24	4.2	175	27.4	22.3	1.38	48.0	
Mol.	0	5.0	135	21.9	77.9	1.26	29.6	23.5
	2	5.4	190	24.2	146.2	1.53	46.0	
	4	5.0	180	25.8	123.0	1.53	46.4	
	6	4.9	140	22.0	86.0	1.24	30.8	
	8	4.8	145	23.9	54.6	1.34	34.7	
	24	4.8	130	24.8	30.6	1.40	32.2	
Ber.	0	3.4	70	29.8	32.5	1.23	20.9	17.0
	2	4.0	70	29.4	139.7	1.43	20.6	
	4	3.3	95	28.9	150.0	1.15	27.5	
	6	3.3	160	28.8	96.7	1.15	46.1	
	8	3.0	130	29.6	42.0	1.08	38.5	
	24	3.2	85	28.9	23.4	1.12	24.6	
Sie.	0	1.4	10	21.8	36.0	0.35	2.2	6.3
	2	1.3	5	22.7	52.0	0.34	1.1	
	4	0.9	20	22.5	38.7	0.23	4.5	
	6	2.0	10	21.8	29.0	0.50	2.2	
	8	1.9	15	21.5	28.6	0.47	3.2	
	24	1.1	10	25.6	24.9	0.33	2.6	
And	0	2.4	70	28.7	19.0	0.83	20.1	24.2
	2	2.6	85	29.9	63.9	0.95	25.4	
	4	1.9	75	29.6	40.0	0.68	22.2	
	6	2.1	60	28.8	28.8	0.73	17.3	
	8	2.8	55	27.6	28.9	0.93	15.2	
	24	1.1	45	28.2	36.5	0.37	12.7	

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Tou.	0	2.0	55	26.5	40.0	0.63	14.6	23.2
	2	1.8	55	30.2	56.5	0.66	16.6	
	4	1.7	55	29.2	47.9	0.66	16.1	
	6	1.9	55	27.3	30.3	0.62	15.0	
	8	2.2	65	26.3	31.1	0.69	17.1	
	24	1.1	45	28.2	36.5	0.37	12.7	
Sch.	0	1.9	15	28.0	35.1	0.64	4.2	6.6
	2	1.6	10	24.8	62.7	0.47	2.5	
	4	1.3	5	26.6	62.2	0.41	1.3	
	6	2.0	5	27.0	42.5	0.64	1.4	
	8	2.3	10	24.7	49.8	0.67	2.5	
	24	2.4	5	26.1	40.6	0.74	1.3	

TABLE E. The parameters during and after TRH infusion in three patients with preclinical primary hypothyroidism.

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Bla.	0	5.5	125	31.8	11.0	2.16	39.8	18.4
	2	6.2	150	31.3	125.2	2.39	47.0	
	4	6.7	180	33.4	146.9	2.80	60.1	
	6	6.7	175	31.8	69.5	2.63	55.7	
	8	5.8	175	31.9	26.8	2.29	55.8	
	24	5.3	105	32.1	5.3	2.11	33.7	
Ren.	0	7.3	140	30.5	18.8	2.73	42.7	15.6
	2	7.6	160	28.2	161.0	2.58	45.1	
	4	8.7	240	31.2	136.0	3.34	74.9	
	6	8.9	205	31.4	124.0	3.44	64.4	
	8	9.4	140	28.5	57.0	3.23	39.9	
	24	8.4	160	28.4	9.4	2.88	45.4	
Lou.	0	5.0	-	23.1	9.0	-	-	-
	2	6.6	-	22.4	116.1	-	-	
	4	7.2	-	25.4	129.0	-	-	
	6	7.3	-	23.1	29.0	-	-	
	8	7.5	-	24.6	19.0	-	-	
	24	6.5	-	24.3	7.5	-	-	

TABLE F. The parameters during and after TRH infusion in two patients with hypothalamic hypothyroidism.

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Bre.	0	5.6	85	24.9	<0.5	1.64	21.2	12.9
	2	6.5	120	25.7	19.3	1.98	30.8	
	4	7.5	125	24.3	9.6	2.13	30.4	
	6	8.9	155	27.3	2.2	2.91	42.3	
	8	6.7	110	24.1	<0.5	1.89	26.5	
	24	6.2	85	23.8	<0.5	1.72	20.2	

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Vee. I	0	4.5	95	24.6	0.9	1.30	23.4	18.0
	2	5.4	175	25.6	17.0	1.63	44.8	
	4	5.7	140	28.7	10.0	1.98	40.2	
	6	5.2	145	25.2	4.2	1.54	36.5	
	8	6.3	175	24.1	1.0	1.77	42.2	
	24	6.0	110	24.6	0.7	1.73	27.1	
Vee. II	0	4.7	80	24.1	2.9	1.32	19.3	14.6
	1/6	-	90	-	-	-	-	
	1/3	-	85	-	-	-	-	
	1/2	-	90	-	-	-	-	
	2/3	-	120	-	-	-	-	
	1	-	125	-	-	-	-	
	2	5.7	125	24.4	17.9	1.63	30.5	
	4	5.8	140	24.2	9.2	1.64	33.9	
	6	6.0	135	23.7	8.3	1.66	32.0	
	8	5.7	100	22.7	4.7	1.50	22.7	
	24	5.8	80	22.8	<0.5	1.53	18.2	

TABLE G. The parameters during and after TRH infusion in patients with various pituitary abnormalities.

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Boo.	0	7.4	60	38.2	0.9	3.67	22.9	6.2
	2	7.5	100	38.9	4.3	3.81	38.9	
	4	8.7	140	41.1	<0.5	4.75	57.5	
	6	7.3	130	38.6	0.8	3.67	50.2	
	8	8.5	125	38.6	<0.5	4.27	48.3	
	24	8.2	60	37.1	0.6	3.91	22.3	
Schi.	0	8.9	175	28.4	1.7	3.05	49.7	16.3
	2	9.8	210	28.8	21.4	3.41	60.5	
	4	10.5	305	29.5	26.9	3.76	90.0	
	6	12.0	275	28.6	11.7	4.14	78.7	
	8	11.9	310	30.1	6.6	4.37	93.3	
	24	11.4	185	29.8	2.0	4.14	55.1	
Zijl. I	0	6.3	190	28.5	2.8	2.17	55.2	25.4
	2	7.9	225	28.5	16.3	2.72	64.1	
	4	8.7	215	30.1	17.6	3.20	64.7	
	6	8.4	250	30.7	7.8	3.16	76.8	
	8	8.6	220	31.0	4.1	3.28	68.2	
	24	8.2	210	29.9	3.8	2.99	62.8	
Zijl. II	0	6.0	95	31.2	0.6	2.30	29.6	12.9
	2	6.0	180	33.3	8.3	2.50	59.9	
	4	7.5	150	32.3	8.0	3.00	48.5	
	6	7.4	145	34.0	1.5	3.16	49.3	
	8	7.6	120	33.9	<0.5	3.23	40.7	
	24	6.9	100	32.4	<0.5	2.78	32.4	

	time	T_4	T_3	RU	TSH	FT_4F	FT_3F	FT_3F/FT_4F
Tem.	0	6.7	135	30.7	<0.5	2.52	41.4	16.4
	2	7.2	170	31.4	4.2	2.79	53.4	
	4	8.4	185	30.4	4.1	3.12	56.2	
	6	7.4	200	29.3	0.9	2.63	58.6	
	8	7.1	155	31.6	<0.5	2.77	49.0	
	24	7.4	130	32.7	<0.5	3.01	42.5	
Nij.	0	5.1	110	26.5	1.9	1.61	29.2	18.1
	2	6.6	135	27.4	8.0	2.16	37.0	
	4	7.0	160	26.3	8.2	2.19	42.1	
	6	6.3	145	26.7	3.9	2.00	38.7	
	8	5.9	150	26.6	1.2	1.87	39.9	
	24	6.1	120	27.1	2.4	1.97	32.5	
Sche.	0	3.2	90	31.3	0.7	1.23	28.2	22.9
	2	3.1	80	30.5	<0.5	1.16	24.4	
	4	3.4	130	28.6	0.8	1.17	37.2	
	6	2.8	125	29.4	1.0	1.00	36.8	
	8	3.3	70	29.4	0.9	1.18	20.6	
	24	3.3	80	28.5	<0.5	1.13	22.8	

TABLE H. Results in patients with autonomous thyroid function after injection of 500 µg TRH.

	age/ sex	min.	T_3	TSH	T_4	RU	FT_4F	FT_3F	FT_3F/FT_4F	thyroid scint.
Kla.	41/M	-30	210	<0.5	13.8	41.3	7.58	86.7	11.4	homog.
		0	220	<0.5						
		20	235	<0.5						
		60	195	<0.5						
		120	220	<0.5						
Omt.	48/F	-30	160	<0.5	15.2	39.2	7.79	60.6	7.8	nodul.
		0	165	0.9						
		20	170	0.5						
		60	175	<0.5						
		120	185	0.8						
Loe.	42/M	-30	180	<0.5	13.1	37.3	6.30	67.1	10.7	homog.
		0	155	<0.5						
		20	145	0.9						
		60	175	<0.5						
		120	180	<0.5						
Tim.	56/F	-30	155	2.1	18.7	34.4	8.11	53.3	6.6	homog.
		0	155	1.0						
		20	160	2.0						
		60	140	1.2						
		120	165	1.3						
Jon.	87/M	-30	145	<0.5	10.0	43.6	5.91	63.2	10.7	multi-nodul.
		0	145	<0.5						
		20	170	<0.5						
		60	170	<0.5						
		120	135	<0.5						

	age/ sex	min.	T_3	TSH	T_4	RU	FT_4F	FT_3F	FT_3F/FT_4F	thyroid scint.
Kel.	18/F	-30	295	0.9	12.3	32.4	4.95	95.6	19.3	homog.
		0	310	1.7						
		20	285	0.7						
		60	315	<0.5						
		120	275	<0.5						

TABLE I. Results of two TRH-tests in normals, using bolus injections of 200 µg 48 hours apart.

	test	min.	T_4	RU	T_3	TSH	FT_4F	FT_3F
Boe.	1	0	11.2	31.7	150	1.1	4.38	47.6
		20	10.9	30.0	235	7.2	3.99	70.5
		60	10.6	29.0	205	4.0	3.72	59.5
	2	0	9.7	29.7	115	1.0	3.51	34.2
		20	10.0	32.2	145	4.6	3.99	46.7
		60	9.9	35.9	190	2.6	4.53	68.2
Hui.	1	0	8.4	36.3	110	2.6	3.90	39.9
		20	8.9	33.3	160	16.1	3.70	53.3
		60	8.8	35.5	170	10.1	3.97	60.4
	2	0	8.1	31.2	115	1.4	3.11	35.9
		20	8.0	31.2	140	10.0	3.07	43.7
		60	8.4	32.3	200	8.1	3.35	64.4
Wol.	1	0	5.6	34.8	70	3.7	2.46	24.4
		20	5.6	35.4	70	19.5	2.52	28.3
		60	6.4	35.5	105	15.5	2.89	37.3
	2	0	4.4	34.1	65	2.2	1.89	22.2
		20	4.8	37.6	75	14.7	2.33	28.2
		60	4.8	37.4	85	11.6	2.31	31.8
Hum.	1	0	6.4	31.8	110	3.1	2.52	35.0
		20	7.8	31.8	150	13.5	3.07	47.7
		60	8.6	33.1	215	10.1	3.55	71.2
	2	0	6.4	31.0	130	2.5	2.43	40.3
		20	6.6	31.6	120	7.5	2.57	37.9
		60	6.9	30.8	130	5.9	2.61	40.0
Vd K.	1	0	7.3	30.6	90	5.9	2.73	27.5
		20	7.9	30.5	125	13.2	2.95	38.1
		60	7.9	30.7	160	10.8	2.97	49.1
	2	0	7.4	28.9	100	3.8	2.59	28.9
		20	7.5	29.0	145	9.5	2.63	42.1
		60	7.3	28.6	150	3.8	2.52	42.9
Kra.	1	0	6.3	32.8	105	4.4	2.57	34.4
		20	6.0	33.5	110	11.6	2.52	36.9
		60	6.8	33.1	115	10.1	2.81	38.1
	2	0	6.0	32.6	95	2.7	2.43	31.0
		20	6.4	33.0	85	9.4	2.63	28.1
		60	6.2	33.6	105	7.9	2.61	35.3

	test	min.	T_4	RU	T_3	TSH	FT_4F	FT_3F
Bak.	1	0	7.0	29.5	115	4.2	2.51	33.9
		20	7.2	30.1	100	17.1	2.64	30.1
		60	7.0	30.0	155	14.1	2.56	46.5
	2	0	6.7	30.0	95	3.1	2.45	28.5
		20	6.9	33.3	85	11.3	2.87	28.3
		60	7.1	31.5	90	10.0	2.76	28.4
Kam.	1	0	8.1	27.3	110	3.2	2.64	30.0
		20	8.1	29.1	75	11.4	2.86	21.8
		60	8.0	28.9	100	9.8	2.80	28.9
	2	0	7.8	28.9	105	3.3	2.73	30.3
		20	8.0	29.7	80	7.6	2.89	23.8
		60	7.8	28.2	110	6.8	2.65	31.0
Pil.	1	0	6.9	32.5	105	3.6	2.79	34.1
		20	6.0	32.9	105	11.5	2.46	34.5
		60	6.3	32.0	105	9.0	2.50	33.6
	2	0	7.3	30.8	105	3.8	2.76	32.3
		20	6.6	31.7	105	10.6	2.58	33.3
		60	7.1	31.8	95	8.4	2.79	30.2
Van R.	1	0	7.2	32.1	95	2.9	2.86	30.5
		20	7.9	32.2	110	8.3	3.15	35.4
		60	7.6	31.8	115	6.7	2.99	36.6
	2	0	7.2	31.4	95	2.9	2.79	29.8
		20	7.4	31.9	95	6.6	2.92	30.3
		60	8.0	32.8	105	4.5	3.27	34.4
Slu.	1	0	8.5	30.7	120	4.2	3.20	36.8
		20	8.8	31.7	115	9.8	3.44	36.5
		60	9.3	31.2	130	8.0	3.57	40.6
	2	0	8.8	30.8	120	2.7	3.32	37.0
		20	8.8	31.7	90	8.2	3.44	28.5
		60	8.9	32.9	130	6.9	3.65	42.8
Van Z.	1	0	8.9	30.2	120	3.9	3.28	36.2
		20	8.6	30.8	130	15.7	3.25	40.0
		60	9.2	29.5	165	11.9	3.30	48.7
	2	0	8.8	29.8	100	2.6	3.19	29.8
		20	8.4	30.3	120	11.1	3.11	36.4
		60	8.5	30.5	125	9.5	3.17	38.1

TABLE J. Corrected percentages of T_3 binding in sera of patient Lou., after addition of different amounts of T_3.

Interval from	Enrichment of the sample in ng/dl by T_3 addition. 0	156	313	625	1250	2500	5000	10000
TRH study								
- 2 days	74.0	74.9	76.1	73.0	65.4	50.1	35.4	
0 hours	78.6	79.2	76.5	71.9	62.7	48.3	32.8	20.7
2 hours	80.2	78.2	76.7	72.8	62.8	48.9	33.5	20.4
4 hours	72.0	71.3	70.5	65.8	57.8	45.6	32.7	
6 hours	71.7	71.1	70.1	65.5	57.7	46.1	34.5	
8 hours	75.0	72.5	69.0	65.3	56.9	45.3	32.5	
24 hours	73.3	72.0	71.4	70.2	67.1	57.1	40.8	24.2
8 weeks	39.3	42.3	43.4	42.2	36.4	26.7	16.5	
19 weeks	26.9	24.8	26.8	25.0	18.5	12.3	7.0	
28 weeks	12.5	12.5	12.2	12.5	8.6	5.2	2.5	
58 weeks	0.1	0.1	0.1	0.5	0	0.2	-0.6	
118 weeks	0.3	-0.1	0.8	1.4	0.5	0.6	0.7	

TABLE K. Corrected percentages of T_3 binding in sera of patient Lou., after addition of different amounts of T_4.

interval from	enrichment of the sample in ng/dl by T_4 addition 0	156	313	625	1250	2500	5000	10000
TRH study								
2 hours	75.9	80.2	79.6	81.0	79.8	79.4	79.3	78.7
24 hours	73.0	74.1	73.2	76.0	76.3	76.3	76.7	73.4
19 weeks	24.9	27.7	26.9	27.5	27.6	27.5	27.2	26.9

TABLE L. Corrected percentages of T_3 binding in sera of patient Juc., after addition of different amounts of T_3 or T_4.

sample	addition	enrichment of the sample in ng/dl. 0	156	313	625	1250	2500	5000	10000
0 hours	T_3	29.8	31.2	29.0	27.8	22.9	16.3	10.0	6.5
	T_4	30.0	29.4	29.2	30.0	30.1	31.2	28.0	29.1
8 hours	T_3	26.7	28.2	27.0	25.9	22.2	16.2	10.0	7.2
	T_4	29.1	29.0	28.6	29.6	26.8	29.4	28.4	28.1

EXPLANATION OF THE SYMBOLS

A	=	functional capacity of the thyrotropic part of the pituitary to secrete TSH.
	=	in model terms: the gain (amplification factor) of the pituitary.
B	=	functional capacity of the thyroid to secrete T_4.
	=	in model terms: the gain (amplification factor) of the thyroid concerning T_4.
b	=	thyroidal secretion ratio of T_3 and T_4, normally 0.10.
bB	=	functional capacity of the thyroid to secrete T_3.
	=	in model terms: the gain (amplification factor) of the thyroid concerning T_3.
C	=	target organ sensitivity for T_3.
N	=	TRH stimulus to the pituitary.
	=	in model terms: the command signal.
E	=	thyroid hormone effect (on the pituitary: inhibition of TSH secretion.
N-E	=	net stimulus to the pituitary to secrete TSH.
	=	in model terms: the error signal.
k_3	=	fractional conversion rate of T_4 to T_3, normally 3% per day.
TT_4	=	total T_4 pool with a volume V_4 = 11 L.
TT_3f	=	T_3 pool in the "fast" compartment with a volume V_3f = 22 L.
TT_3s	=	T_3 pool in the "slow" compartment with a volume V_3s = 18 L.
TLG	=	total loop gain, the product of the gains of the pituitary and the thyroid.
	=	response of the control system, expressed as T_3-equivalents per day, to a certain TRH stimulus.